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VOL. 16, SEC. C JANUARY, 1938 NUMBER 1

CORRELATION BETWEEN YIELD AND PROTEIN CONTENT OF WHEAT AND BARLEY IN RELATION TO BREEDING[1]

BY K. W. NEATBY[2] AND A. G. MCCALLA[3]

Abstract

High yielding varieties of wheat and barley have a marked tendency to be constitutionally low in protein content. While varieties characterized by moderately high yield and high protein content are known, it is doubtful whether the maximum possible yield can be combined with maximum possible protein. The problem of breeding hard red spring wheats is complicated by this relation, while breeding soft wheats and low protein malting barleys is simplified.

Introduction

Regardless of whether cereals are bred for human consumption or as food for livestock, yield and protein content are characters of great importance. These two characters, in common with many others, are difficult to manipulate in breeding work because they are inherited in a complex manner, and their expression is notoriously subject to environmental influence. There is a general tendency for conditions conducive to high yield to depress protein content, and for those which result in low yield to increase it. Exceptions to this general rule do occur, however, particularly when different regions or soil types are included in the comparisons made. A study of the relation between yield and protein content of Red Bobs and Marquis wheat at Edmonton was made by Malloch and Newton (1). In 1930 and again in 1931, 50 rod-row samples were taken from one wheat field, and yield and protein determinations made. The relation as expressed by the correlation coefficient was $-.68$ for Red Bobs in 1930 and $-.42$ for Marquis in 1931.

Another illustration of the principle suggested by the results of Malloch and Newton is afforded by the rod-row tests of rust resistant hybrids conducted at the Dominion Rust Research Laboratory, Winnipeg, from 1932 to 1936 inclusive. In Fig. 1 the mean yield is plotted against the mean protein for each year. Detailed data are recorded in Table II. But for the year 1933, a very close negative relation would be indicated. However, the 1933 results must be disregarded since the protein determinations were not made on the rod-row material which provided the yield data, but on increase plots grown on a field about half a mile away from the rod-row test. In the other four years both protein and yield data were derived from the same plots.

[1] *Manuscript received October 2, 1937.*
Contribution from the Department of Field Crops, University of Alberta. Issued as paper No. 129 of the Associate Committee on Grain Research of the National Research Council and the Dominion Department of Agriculture.

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An examination of yield and protein data secured from cereal variety trials over a period of years at the University of Alberta suggested to the authors that genetical differences in the yielding ability of varieties might be associated with protein content. If varieties characterized by high yield tend to be low in protein, the improvement of malting barley and soft wheat by breeding methods would be simplified, but breeding hard red spring wheats would be seriously complicated. The only published data bearing on this question which have come to the attention of the authors are those of Waldron (2).* He found the correlation coefficient calculated from the yield and protein content of 25 varieties grown in a comparative test to be $-.556$.

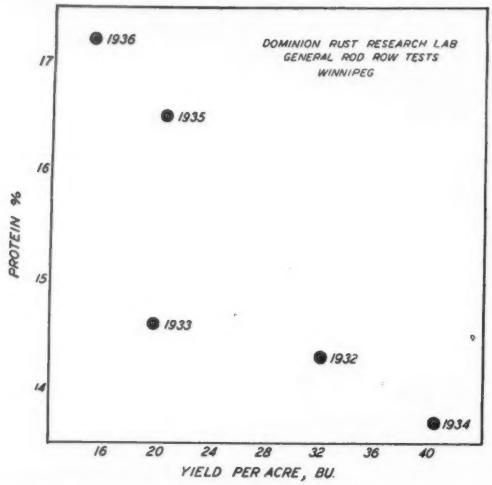

FIG. 1. *The relation between yield per acre and protein content of wheat at Winnipeg for the period 1932 to 1936.*

The publication of the data assembled in this paper has been made possible through the kind permission of the following: Dr. B. B. Bayles, Bureau of Plant Industry, United States Department of Agriculture; Dr. L. H. Newman, Cereal Division, Dominion Department of Agriculture; Drs. C. H. Goulden and R. F. Peterson, Dominion Rust Research Laboratory; and the Associate Committee on Field Crop Diseases of the National Research Council and the Dominion Department of Agriculture.

Hard Wheats

The co-operative test of rust resistant varieties of wheat conducted by the Associate Committee on Field Crop Diseases has been located at various points in each of the three prairie provinces for several years. Yield and

**The results published by A. G. O. Whiteside (Can. J. Research, C, 14: 387-393. 1936) were unfortunately overlooked when this paper was prepared.*

protein data for the years 1933, 1934 and 1936 are summarized in Table I. In this and other tables, yields are expressed in terms of bushels per acre, and protein contents are calculated on the basis of a moisture content of 13.5%. The correlation coefficients vary from −.07 to −.83 in the 1933 series. At certain stations, notably Morden and Saskatoon, the relation between high yield and low protein content is very close. In 1933 each variety was seeded in four replicate plots. Under such circumstances observed differences in yield may not be entirely genetical, since identical conditions for all varieties cannot be secured. With this fact in mind the correlation between the general means of yield and protein for all stations was determined. The coefficient obtained, −.55, is almost certainly due largely to genetical causes. It is true that environmental effects cannot be entirely eliminated, since a certain degree of interaction between variety and station for each of the two characters in question is likely to occur. The relative importance of genetical and environmental effects on the correlation coefficient cannot be determined without knowledge of the protein content of individual plots. This information is, unfortunately, lacking.

The practical significance of the above results is indicated by the regression coefficients and the figures in the last four columns in the table. It is clear that if selection was based on yield alone the general level of protein content would be lowered. Similarly, if protein content was emphasized, the selection would automatically favor low yield. Swan River is the only station at which the relation fails to hold. The figures based on the 1933 general means are very significant. The difference of 7.9 bushels per acre between the five varieties with the highest protein and the five with the lowest is, from a breeding point of view, of substantial importance.

The data for 1934 are similar, though the relations are not quite so close. In 1936 the magnitude of the correlation coefficients was greatly reduced. This reduction is without a doubt partly due to the fact that protein content has been stressed in the choice of varieties for inclusion in the co-operative test. An examination of the records has revealed the fact that several varieties which were outstanding from the point of view of yield during the period 1931–33 were discarded on account of low protein and inferior loaf volume. It is necessary to point out, however, that the 1936 results are not strictly comparable with those of 1933 and 1934, since in 1936 the mean yield was low and the mean protein content high, owing to unusually dry conditions.

The results of three spring wheat and two winter wheat tests are summarized in Table II. In the University of Alberta tests, the correlation coefficients do not indicate a very close relation between protein content and yield, except in the case of the 1930 results. However, in all years but 1936 the differences in yield between the five varieties with highest and the five with lowest protein are considerable. Likewise, the difference in protein content of the five highest yielding and the five lowest yielding varieties are large enough to deserve careful attention.

TABLE I

RELATION BETWEEN YIELD AND PROTEIN, CO-OPERATIVE TEST OF RUST RESISTANT VARIETIES

Place	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
			Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
1933. 36 varieties										
Edmonton, Alta.	65.7	15.2	10.8	5.3	−0.64	−0.073	57.7	68.4	14.6	16.2
Fallis, Alta.	43.8	12.4	15.0	8.0	− .50	− .076	33.0	45.4	12.0	13.5
Lacombe, Alta.	53.5	15.8	11.6	4.1	− .71	− .073	44.2	60.1	15.3	16.6
Indian Head, Sask.	50.9	15.6	10.3	4.6	− .58	− .081	44.7	56.1	15.0	16.3
Saskatoon, Sask.	16.5	15.6	14.2	4.3	− .78	− .221	13.6	19.4	14.9	16.5
Scott, Sask.	6.9	14.9	21.0	4.2	− .43	− .186	5.2	7.9	14.6	15.2
Swift Current, Sask.	22.5	15.8	12.1	4.6	− .15	− .040	22.8	24.3	15.2	15.8
Brandon, Man.	37.2	14.4	10.2	5.2	− .45	− .088	36.5	37.5	14.1	14.8
Morden, Man.	27.1	16.4	12.9	4.4	− .83	− .171	21.5	31.9	15.5	17.2
Swan River, Man.	42.4	11.0	14.4	12.7	− .07	− .015	44.5	43.8	11.6	11.6
Winnipeg, Man.	17.1	14.1	11.7	5.7	− .71	− .282	14.7	19.0	13.6	14.8
Mean	33.7	15.4	9.0	4.2	− .55	− .118	29.6	37.5	14.8	16.5
1934. 25 varieties										
Edmonton, Alta.	53.6	14.9	9.4	3.7	− .10	− .011	51.9	60.1	14.8	15.1
Fallis, Alta.	18.4	9.7	13.9	4.7	− .77	− .175	15.6	21.3	9.0	10.2
Lacombe, Alta.	26.5	15.0	13.6	5.0	− .68	− .143	22.9	32.7	14.2	15.7
Indian Head, Sask.	32.6	14.9	11.2	4.1	− .31	− .052	30.6	35.8	14.4	15.5
Saskatoon, Sask.	26.7	15.9	8.0	2.9	− .30	− .065	26.6	27.9	15.9	16.0
Swift Current, Sask.	20.0	16.9	7.9	2.8	+ .11	+ .034	20.2	19.9	17.1	16.9
Brandon, Man.	33.9	15.1	7.7	3.5	− .50	− .100	31.7	35.8	14.8	15.5
Morden, Man.	34.0	16.1	6.9	3.4	− .43	− .100	32.4	36.4	15.7	16.4
Swan River, Man.	36.3	10.0	10.8	8.9	− .07	− .015	33.9	37.4	9.7	10.0
Solsgirth, Man.	24.5	13.6	13.7	3.8	− .31	− .049	24.3	27.1	13.1	13.6
Mean	30.3	14.1	5.7	3.4	− .50	− .136	29.3	30.9	13.8	14.6

TABLE I—*Concluded*

RELATION BETWEEN YIELD AND PROTEIN, CO-OPERATIVE TEST OF RUST RESISTANT VARIETIES—*Concluded*

Place	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
			Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
1936. 22 varieties										
Edmonton, Alta.	30.4	15.2	11.7	6.0	− .39	− .100	28.4	31.4	14.4	15.3
Fallis, Alta.	10.0	15.1	18.4	5.8	− .64	− .308	8.3	11.1	14.8	16.2
Lacombe, Alta.	21.7	16.0	19.3	4.2	− .14	− .023	19.6	22.7	16.0	15.8
Indian Head, Sask.	28.6	15.7	10.2	5.4	+ .11	+ .032	28.7	28.0	15.5	15.7
Saskatoon, Sask.	18.4	18.2	11.3	4.3	− .38	− .141	17.2	19.5	18.1	18.6
Scott, Sask.	6.8	17.3	11.6	4.2	− .35	− .320	6.5	7.4	16.9	17.7
Swift Current, Sask.	10.4	18.8	10.3	3.1	+ .23	+ .126	10.8	10.8	18.9	18.8
Brandon, Man.	26.0	16.6	12.0	4.4	.00	.005	27.0	28.0	16.7	16.7
Morden, Man.	17.3	19.8	11.7	3.8	− .37	− .138	16.8	18.8	19.1	20.1
Solsgirth, Man.	11.8	16.2	13.1	6.0	− .61	− .381	10.3	12.7	15.8	17.2
Winnipeg, Man.	10.1	16.5	22.8	4.9	− .10	− .035	10.7	10.2	16.7	17.1
Mean	17.9	16.8	6.7	3.1	− .25	− .108	17.6	18.4	16.6	17.0

TABLE II

RELATION BETWEEN YIELD AND PROTEIN, MISCELLANEOUS HARD WHEATS

Place	Year	No. of varieties	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
					Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
Rod-row tests, University of Alberta												
Edmonton	1929	33	29.9	16.1	17.5	7.7	−0.65	−0.154	23.9	34.1	15.0	17.9
Edmonton	1930	21	35.6	15.6	16.0	7.1	− .76	− .147	30.3	40.6	15.0	16.9
Edmonton	1931	49	52.3	14.5	12.4	6.3	− .54	− .075	42.3	54.0	14.1	15.9
Edmonton	1932	67	43.2	15.1	10.5	5.3	− .38	− .067	39.9	46.0	14.8	15.8
Edmonton	1933	35	61.3	15.5	10.6	5.4	− .30	− .039	58.2	65.3	15.4	16.1
Edmonton	1936	15	40.8	14.4	13.6	5.5	− .34	− .048	39.2	39.4	13.7	14.6
Fallis (fallow)	1933	12	39.1	11.5	22.4	6.8	− .34	− .031	35.7	39.1	11.2	11.8
Fallis (stubble)	1933	12	18.0	10.5	14.8	8.4	− .60	− .197	17.0	19.2	10.0	10.6
Rust resistant hybrids, Dominion Rust Research Laboratory												
Winnipeg	1932	101	32.0	14.3	11.7	5.1	− .37	− .072	29.7*	35.0*	13.4*	14.6*
Winnipeg	1933	141	19.6	14.6	13.3	5.4	− .42	− .127	17.0*	21.8*	14.0*	14.8*
Winnipeg	1934	124	40.4	13.7	11.2	5.5	− .23	− .038	37.0*	40.8*	13.0*	14.1*
Winnipeg	1935	108	20.4	16.5	19.8	5.3	− .19	− .040	16.3*	20.4*	15.6*	16.3*
Winnipeg	1936	85	15.0	17.2	14.3	4.2	− .16	− .052	12.9*	15.5*	16.9*	17.4*
Drought hybrids and miscellaneous varieties, University of Alberta												
Brooks, Alta.	1936	75	10.0	12.0	24.7	8.0	− .51	− .138	7.5*	11.8*	11.6*	13.0*
Winter wheats, University of Alberta												
Edmonton	1931	46	28.4	15.8	16.9	3.5	− .26	− .031	24.0	31.8	15.4	15.7
Winter wheats, U.S.D.A. western region												
**5 Station mean	1935	24	52.2	9.3	5.7	4.6	− .57	− .081	48.7	54.4	8.9	9.7

* *10 samples instead of 5.*
** *Pullman, Pomeroy and Walla Walla, Washington; Pendleton, Oregon; Newton, Utah.*

Perhaps the most interesting feature of the results of the Dominion Rust Research Laboratory tests is the reduction in magnitude of the coefficients from 1932 to 1936. It is quite possible that this is due, in part at least, to the elimination of high yielding strains on the basis of protein content and of high protein strains on account of low yield.

The test of miscellaneous varieties and hybrid strains conducted at Brooks, Alberta, by the University in 1936 was exposed to conditions of very severe drought. The relation between yield and protein is similar to those already described.

Numerous data on winter wheat varieties are available, but tests in which winter killing occurred cannot be legitimately quoted in the present connection. The data at the bottom of Table II suggest that the relation observed in spring wheats holds also in winter wheats. In the United States Department of Agriculture test, samples from five stations were composited for protein determinations. This procedure virtually eliminates environmental effects and, consequently, the correlation coefficient of $-.57$ is almost entirely due to genetical relations. The magnitude of the regression coefficient indicates, however, that fairly large differences in yield are associated with relatively small differences in protein content.

Early Hard Spring Wheats

The development of early high yielding spring wheats of good quality is engaging the attention of several Canadian plant breeders. It so happens that the areas of Manitoba, Saskatchewan and Alberta chiefly concerned are, in many cases, characterized by relatively infertile soils and more humid conditions than those prevailing on the open plains. Therefore the question of yield in relation to quality assumes considerable importance. A new variety, if it is to be accepted, must be productive and it must be early. Protein content is equally important because the conditions of soil and climate in the northern areas, generally speaking, are not conducive to the production of wheat of high protein content.

The situation with regard to yield and protein content in several comparative trials including new and old early varieties is summarized in Table III. Again there is a definite and, in some cases, a close inverse relation between yield and protein content. In the University of Alberta test, 18 of the 32 varieties were selected from the cross, Reward × Double Cross. The latter variety was selected by Dr. H. K. Hayes of the University of Minnesota from (Marquis-Iumillo) × (Marquis-Kanred). The necessity of compromising between maximum yield and maximum protein is well brought out by the Edmonton test of Reward × Double Cross strains. Selection for maximum yield will almost certainly involve a sacrifice of protein.

The Fallis results are of particular interest, since the soil at Fallis is deficient in nitrogen and sulphur and is typical of large areas for which early varieties are being developed. The high values of the regression coefficients calculated

TABLE III

RELATION BETWEEN YIELD AND PROTEIN, EARLY WHEATS, 1936

Place	No. of varieties	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{vp}	b_{pv}	Yield of		Protein of	
				Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
University of Alberta, general series											
Edmonton	32	27.5	14.6	12.2	5.4	−0.35	−0.082	25.2	29.3	13.8	14.7
Fallis	32	8.8	14.8	23.3	7.0	− .54	− .272	6.6	10.3	14.0	15.6
University of Alberta, Reward × Double Cross											
Edmonton	18	27.7	15.5	13.0	4.4	− .70	− .176	26.1	32.1	14.7	16.0
Fallis	18	9.5	14.9	13.4	6.1	− .60	− .323	8.5	10.0	14.5	15.7
Cereal Division co-operative early wheat test											
Beaverlodge	24	42.3	13.3	14.7	7.2	− .54	− .082	37.0	47.9	12.4	13.6
Edmonton	24	25.2	15.4	8.0	7.0	− .40	− .212	24.5	26.8	14.5	15.8
Indian Head	23	23.3	14.3	13.3	8.8	− .56	− .227	21.6	25.9	13.3	15.2
Scott	24	8.6	17.1	9.3	4.6	− .57	− .559	8.1	10.0	16.2	17.7
Swift Current	24	13.6	18.9	5.2	3.1	− .48	− .399	13.0	14.1	18.7	19.4
Ottawa	23	26.7	14.7	11.8	8.1	− .14	− .053	26.6	28.4	14.2	14.8
Mean	22	21.9	15.6	7.2	5.0	− .52	− .257	21.1	22.8	14.8	16.1

on the Fallis tests indicate that the introduction of more productive varieties may result in a more or less serious reduction in protein content.

The same general conclusion can be drawn from the results of the co-operative early wheat test conducted by the Cereal Division of the Dominion Department of Agriculture. The Beaverlodge results are of particular interest, in that the five varieties with the lowest average protein yielded 10.9 bushels per acre more than did the five varieties with the highest average protein content.

Soft Spring Wheats

With a view to determining the adaptability of soft wheats to the gray wooded soil areas of northern and northwestern Alberta, comparative tests of 11 varieties have been conducted at various points by the Department of Field Crops of the University of Alberta. The 1936 results relevant to the subject of this paper are given in Table IV. At every station except Beaverlodge the negative correlation between yield and protein content is high. The low figure for Beaverlodge is undoubtedly due to the fact that varietal differences in yield were small. This is indicated by the low coefficient of variability. The correlation between mean yield and mean protein is extremely high and indicates that within the limits of this experiment the genetical potentialities for protein content are largely determined by the yielding ability of the varieties. It is necessary to point out that two of the varieties, Marquis and Red Bobs, are not soft wheats. However, the position of these two varieties in Fig. 2 indicates that they do not contribute more to

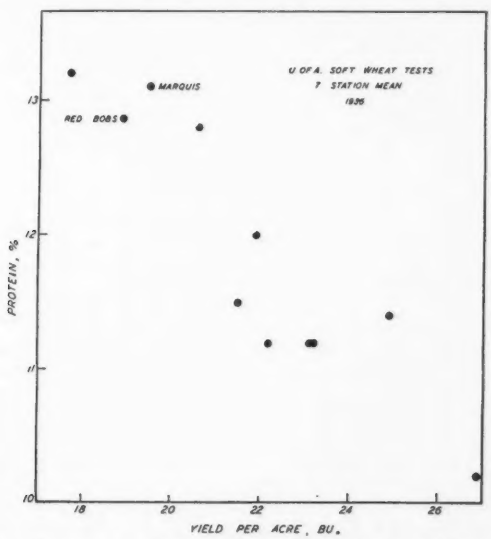

FIG. 2. *The distribution of varieties of soft wheat according to yield per acre and protein content.*

TABLE IV

RELATION BETWEEN YIELD AND PROTEIN, SOFT WHEATS

Place	Year	No. of varieties	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
					Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
University of Alberta tests												
Athabasca	1936	11	6.7	10.2	19.2	10.7	−0.77	−0.660	6.0	7.6	9.4	10.9
Bon Accord	1936	11	9.2	13.7	42.0	9.8	− .86	− .301	6.6	11.9	12.9	14.6
Beaverlodge	1936	11	58.8	11.2	6.7	9.5	− .32	− .086	56.5	59.7	10.7	11.9
Edmonton	1936	11	39.4	13.6	14.8	5.4	− .76	− .121	34.9	43.5	13.0	14.2
Fallis	1936	11	12.8	11.6	20.8	9.5	− .83	− .345	10.8	15.2	10.7	12.5
Mellowdale	1936	11	13.4	9.3	11.7	10.7	− .73	− .456	12.2	14.7	8.5	10.2
Warburg	1936	11	11.8	13.5	20.1	8.9	− .83	− .420	9.6	13.6	12.6	14.5
Mean	1936	11	21.8	11.9	11.7	8.3	− .92	− .357	19.7	23.9	11.0	12.7
U.S.D.A. western region												
Davis, California	1935	15	56.0	9.5	8.0	9.4	− .14	− .028	57.7	54.5	9.8	9.8
1. Three-station composite*	1935	32	59.9	10.8	8.0	8.1	− .56	− .104	53.9	61.0	10.4	11.9
2. Four-station composite*	1935	19	35.7	11.7	7.1	6.9	− .63	− .201	33.0	37.1	11.2	12.2

**1. Aberdeen, Idaho; Logan, Utah; Bozeman, Montana (Irrigated).*

**2. Pullman, Pomeroy and Walla Walla, Washington; Pendleton, Oregon.*

TABLE V

RELATION BETWEEN YIELD AND PROTEIN, UNIVERSITY OF ALBERTA BARLEY TESTS

Place	Year	No. of varieties	Mean yield, bu. per acre	Mean protein, %	Coefficient of variability		r_{yp}	b_{py}	Yield of		Protein of	
					Yield	Protein			Five highest protein	Five lowest protein	Five highest yield	Five lowest yield
Edmonton	1932	22	45.9	15.1	19.8	8.1	−0.45	−0.060	37.0	52.1	14.6	16.1
Edmonton	1933	22	37.9	14.6	15.7	5.1	− .30	− .037	37.4	42.7	13.7	15.0
Edmonton	1934	26	58.7	15.0	18.9	11.3	− .75	− .115	48.1	68.9	13.4	16.6
Edmonton	1936	32	34.9	14.5	17.9	7.3	− .41	− .070	26.1	38.3	14.5	15.7
Fallis	1933	15	24.1	10.0	20.7	8.9	− .80	− .144	20.7	29.6	9.1	10.6
Fallis	1934	12	28.5	8.8	31.1	10.7	− .72	− .077	23.4	36.0	8.1	9.7
Athabasca	1936	9	8.6	10.2	18.1	10.3	− .39	− .249	7.5*	9.0*	9.4*	10.2*
Beaverlodge	1936	10	27.2	12.8	13.7	8.6	− .74	− .219	25.9	28.4	12.3	13.3
Bon Accord	1936	10	12.1	12.8	35.1	10.7	− .21	− .068	11.5	12.7	12.8	12.8
Fallis	1936	10	13.4	12.1	16.2	8.2	− .73	− .330	12.3	14.6	11.8	12.5
Mellowdale	1936	10	11.8	10.2	18.3	10.5	− .66	− .328	10.9	12.7	9.8	10.5
Warburg	1936	10	12.0	13.8	20.9	7.0	− .88	− .342	10.2	13.7	13.1	14.6
Mean	1936	9	14.2	11.9	14.6	7.8	− .72	− .323	13.3*	15.5*	11.4*	12.6*

* *4 varieties instead of 5.*

the correlation than do the other varieties. It is interesting that at all stations except Edmonton and Beaverlodge the plots were located on soil characterized by a deficiency of nitrogen, and the regression coefficients are high. Since it is on these soils that the problem of low protein is acute, the practical importance of a negative relation between yield and protein content is obvious.

Except in the case of Davis, California, the United States Department of Agriculture data (Table IV) indicate the same general relation.

Barley

The data in Table V indicate that the relation in the case of barley is almost as close as that observed in the soft wheats. The 1934 Edmonton test is particularly striking. The five varieties with the lowest average protein content yielded 20.8 bushels more per acre than did the five varieties with the highest protein. The difference in protein content of the five highest- and the five lowest-yielding varieties is equally marked.

In the Fallis tests the differences in yield between the high and low protein varieties are relatively enormous.

Only ten varieties were included in the tests conducted at country points. One variety, Regal, was omitted at Athabasca and, consequently, the comparisons made for that point and for the means include only nine varieties; only the four highest and the four lowest, according to yield, and similar numbers, according to protein content, were used in obtaining the figures in the last four columns. The results of these tests are similar to those obtained at Edmonton. The negative relation between yield and protein content is quite apparent, and the difference in yield between the high and low protein varieties is remarkably consistent from one station to another.

Discussion

In consideration of the foregoing facts, it is certain that there is a genetically controlled association between high yield and low protein content in wheat and barley. The correlation coefficient cannot be used safely as an absolute measure of relationship, which may, in some cases, be non-linear, and the reliability of the coefficients may be tempered by the "grouping" of varieties within the swarm. In order to determine the exact degree of relationship, it will be necessary to plan experiments with that particular purpose in mind. It is probable that the degree of association will vary from one cross to another. Despite the inadequacy of the available data from the point of view of the degree of association, its significance in relation to breeding problems is obvious. This is emphasized by the magnitude of the regression coefficients in most of the tests and, in several cases, by the large differences in protein content between the highest and lowest yielding varieties.

In so far as hard red spring wheats are concerned, the aim is maximum yield and maximum protein content. Efforts to attain this end are based on the assumption that high yield and low protein content are not inseparably

associated. That this assumption is justified is indicated by the performance of Thatcher wheat, for example, which is characterized by relatively high yield and high protein content. In consideration of all available facts it seems probable that genes, the direct effect of which is to stimulate yielding ability, indirectly depress the protein content just as environmental factors which promote high yield frequently do so at the expense of protein content. It is probable, however, that numerous genes that promote high protein in the grain are available, and such genes would, theoretically, tend to offset the depressing effect on protein content of the "high yield" genes. If this is the true state of affairs, the importance of exploring the maximum possible number of different crosses is clear.

To illustrate the varied potentialities of different crosses, a comparison has been made (Fig. 3) between Pentad × Marquis and H-44-24 × Reward. These data are taken from the general rod-row test conducted at the Dominion Rust Research Laboratory. A general negative relation between protein content and yield is apparent in Fig. 3, A, B, C, and D. The lines of the cross H-44-24 × Reward (circles) have a tendency to fall in the high-protein

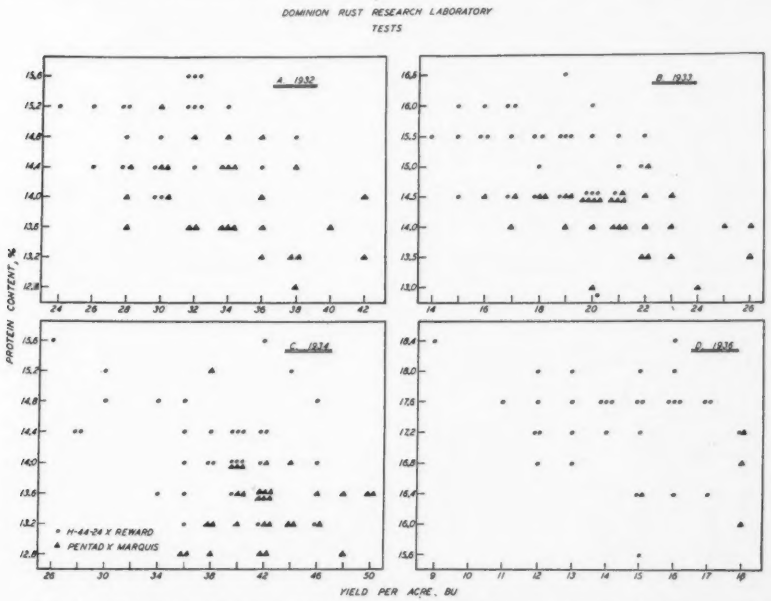

FIG. 3. *The relation between yield per acre and protein content in lines of the crosses H-44-24 × Reward and Pentad × Marquis.*

low-yield area, while the Pentad × Marquis lines (triangles) tend to be high in yield and low in protein. By 1936 (Fig. 3, D) all Pentad × Marquis lines except five (protein data were available for four only) had disappeared from

the test. Doubtless low protein content was an important factor in their disappearance.

To summarize this phase of the discussion it is suggested that the genetical constitution of a high-protein wheat variety may be due to an accumulation of "high protein" genes, or to a paucity of "high yield" genes accompanied by moderate potentialities for high protein.

Throughout this paper it has been assumed that yield and protein content are subject to the same laws of inheritance as are other more readily observable characters. This assumption will hardly be questioned. The differences in general level of yield and protein content for the same material between

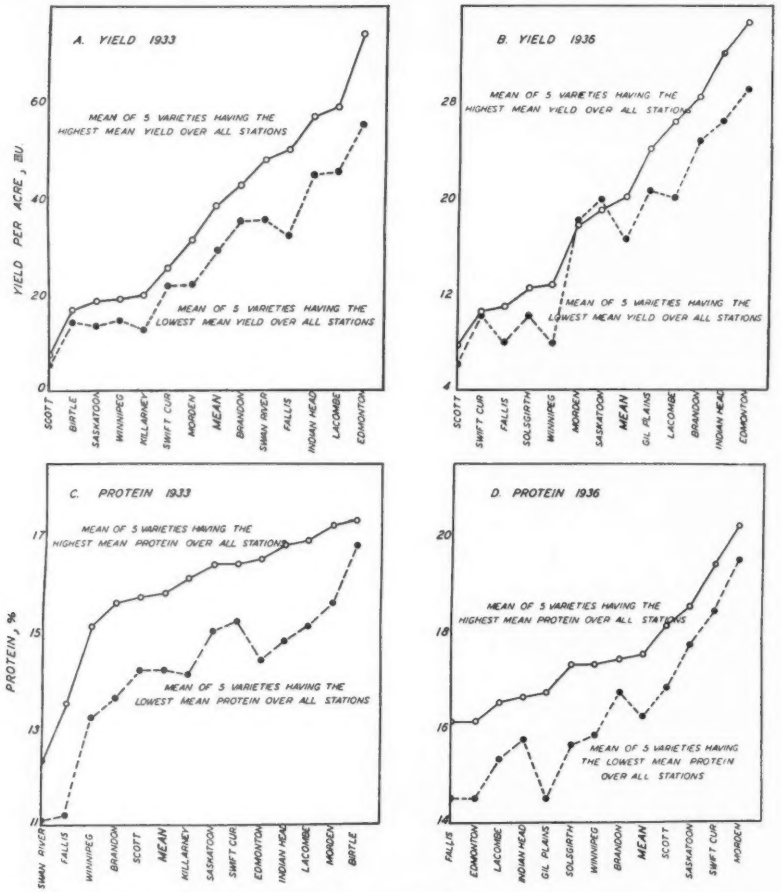

FIG. 4. *The constancy of differences in yield and protein content of wheat varieties at various stations (data from co-operative tests of rust resistant varieties).*

stations are, of course, due to environmental influences such as moisture, soil nitrogen, and general nutritional conditions. The genetical tendency of one variety in the series to be high yielding or high in protein may be so modified by environmental influences that the actual yield or protein content is very low. These influences on the physiology of the plant are not to be confused with the genetical influences on the physiology, since, in any series, the high yielding varieties tend to be relatively high yielding over a wide range of environmental conditions. This is well illustrated by the graphs in Fig. 4, in which the constancy of the genetical differences of rust-resistant varieties from one geographical location to another is shown. If the interaction of these characters with location is very great, then the significance of determinations made at one point may be nullified in so far as other points are concerned. In Fig. 4, A the five varieties giving the highest mean yield for all stations, and the five giving the lowest mean yield, are arranged according to the respective mean values for individual stations in 1933. A similar illustration of the 1936 results is given in Fig. 4, B. The fact that the lines do not cross, except at two stations in 1936, indicates that there is a general agreement between the yield results from station to station. This holds, of course, only in a general way since it is well known that some varieties are more sensitive to regional differences than are others.

The protein data have been arranged in a similar manner (Fig. 4, C and D). The differences are remarkably constant from station to station. This suggests that selection for protein content can be carried out with confidence at any one station. It is possible, however, that certain varieties, or crosses, may behave in an irregular manner under special conditions.

References

1. MALLOCH, J. G. and NEWTON, R. The relation between yield and protein content of wheat. Can. J. Research, 10 : 774-779. 1934.
2. WALDRON, L. R. Yield and protein content of hard red spring wheat under conditions of high temperature and low moisture. J. Agr. Research, 47 : 129-147. 1933.

AGRICULTURAL METEOROLOGY: CORRELATION OF AIR TEMPERATURES IN CENTRAL AND SOUTHERN ALBERTA AND SASKATCHEWAN WITH LATITUDE, LONGITUDE AND ALTITUDE[1]

By J. W. Hopkins[2]

Abstract

Linear partial regression coefficients of the 18-year average (1917–34) monthly mean air temperature recorded at 43 points in central and southern Alberta and Saskatchewan on latitude, longitude, and altitude were determined for each month of the year. The three series of coefficients each show an independent seasonal trend. The decrease in air temperature with altitude is greatest in summer and least in winter, whereas the gradient associated with longitude is most pronounced in winter and least in evidence in summer. The influence of latitude is likewise most pronounced in winter, but shows two minima, in spring and autumn respectively. The monthly regression equations account for most of the variance of the station averages, and hence provide a reasonably satisfactory graduation of the climatological temperature gradients characteristic of this area at different seasons of the year.

These regression equations could not, however, be applied satisfactorily to the monthly averages for individual years, owing to greater local variation. Additional equations were therefore determined from the records for 1935 at 27 stations in the sub-area bounded by the 50th and 52nd parallels and the 104th and 108th meridians. The results suggest that further additions to the number of stations would still be desirable, and that if this was effected a fairly accurate graduation should be possible within this district, even in individual years.

Introduction

According to Irwin (3, pp. 269 *et seq.*) the question of the adequacy of the number of meteorological stations in any area has been subject to little critical study. Irwin points out that the number of stations per square mile, or its reciprocal, "is really quite an inadequate criterion, for in some regions of a given size weather conditions will be almost uniform, in other regions of the same size they will vary greatly."

One aspect of this subject was touched upon in a previous study by the present writer (2), in the course of which the irregular variation (as distinguished from consistent differences between years and between places) in the monthly totals of precipitation recorded at meteorological stations in central and southern Alberta and Saskatchewan was determined, and the theoretical number of stations required to reduce such irregular or random variation in the district averages to specified levels computed.

With respect to the number of stations required in any area to provide a satisfactory indication of positional effects, Irwin (3) suggests that "Perhaps the only adequate criterion is that any meteorological variate (say rainfall) in which we are interested should in any one station be capable of prediction from the values of the same variate at other neighboring stations. For this purpose it is convenient if areas can be selected sufficiently small for the

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regression of the variate on latitude, longitude and altitude to be effectively linear within each such area, and with a sufficient number of stations within the area to enable the regression line to be calculated with an assigned accuracy." A rather different meteorological application of the method of linear regression has recently been made by Schumann (6) in determining the average increase in error of interpolation of monthly rainfall values in South Africa with increasing distance of the point of interpolation from the control stations. His treatment does not, however, take into account the co-ordinates of position and altitude.

In considering the method outlined by Irwin, a distinction may be made between two different types of meteorological data: (a) Climatological averages, specifying the average weather conditions over a period of years; (b) Records descriptive of the weather within the given area during a specified interval of time, such as a particular growing season or portion thereof. Within any climatic zone, (a) may be expected to be more stable than (b), and hence to be capable of specification to an equal degree of accuracy by a coarser network of stations.

In the following sections of this paper, the method is applied to the mean monthly air temperatures at stations in central and southern Alberta and Saskatchewan, which from the meteorological point of view may be expected to provide a homogeneous area, since they are free from major physiographic barriers or large bodies of water which might affect the atmospheric circulation. Linear correlations are determined for the 18-year averages, 1917–34, for each month, permitting a numerical summary of the influence of position and altitude on mean air temperature within the area. The agreement between the actual and linearly graduated values then provides a criterion of the homogeneity of the area represented and of the adequacy of the meteorological network in specifying the temperature characteristics of the area. Finally, the additional variability encountered in considering the results for an individual season is examined quantitatively.

Observational Data

The temperature data used were in all cases extracted from the Monthly Record published by the Meteorological Service of Canada (5). A series of 43 stations in the area designated was found to have continuous or nearly continuous records for the 18-year period 1917–1934. In the earlier years, some gaps occurred, which were filled by the substitution of observations taken at neighboring stations, not otherwise included in the series. Fig. 1 shows the location of the 43 stations, the latitude, longitude and altitude of which will be found in Table I. Table II gives the 18-year average of mean temperature (*i.e.*, mean of daily maximum and minimum) by calendar months for each station and also, at the foot of the columns, the average and standard deviation of the values for all 43 stations for each month. The means illustrate the well-known annual progression of temperature from a minimum, in this case of 8.0° F., in January to a maximum of 63.7° in July. It is to be

FIG. 1. *Location of meteorological stations providing 18-year temperature averages.*

noted that the standard deviation, indicative of the differences between stations in respect of the 18-year average of monthly mean temperature, also shows a definite seasonal trend, being greatest in winter and least in spring and autumn.

Analysis of Climatological Series

From the data in Tables I and II, the partial regression coefficients b_1, b_2 and b_3 of mean temperature on latitude, longitude and altitude were determined by the method of Least Squares. The Normal Equations to determine b_1, b_2 and b_3 for January were:

$$\begin{aligned} 251{,}075\, b_1 + \quad 49{,}761\, b_2 - \quad 1{,}041{,}367\, b_3 &= -11{,}548.5 \\ 49{,}761\, b_1 + 2{,}455{,}745\, b_2 + \quad 5{,}852{,}747\, b_3 &= \quad 50{,}976.2 \\ -1{,}041{,}367\, b_1 + 5{,}852{,}747\, b_2 + 24{,}320{,}901\, b_3 &= \quad 173{,}459.6 \end{aligned}$$

TABLE I

LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS

Station	Latitude north of 49th parallel, min.	Longitude west of 101st meridian, min.	Height above sea level, ft.
Alberta			
Alix	203	730	2585
Bassano	107	688	2625
Calgary	122	782	3540
Calmar	255	770	2200
Edmonton	273	750	2158
Gleichen	112	723	2952
Harmattan	165	803	3500
High River	95	772	3394
Hillsdown	192	755	2940
Lacombe	208	764	2783
Lethbridge	43	711	2961
Lundbreck	34	788	3918
Macleod	44	744	3128
Medicine Hat	61	577	2144
Olds	165	785	3413
Pekisko	82	807	4721
Perbeck	178	725	2850
Ranfurly	269	638	2250
Strathmore	123	743	3160
Saskatchewan			
Anglia	154	430	1861
Battleford	221	440	1620
Chaplin	88	340	2202
Fort Qu'Appelle	107	168	1600
Indian Head	88	160	1924
Kamsack	154	54	1445
Klintonel	38	473	3500
Melfort	232	216	1518
Moose Jaw	81	275	1860
Muenster	192	240	1888
Nashlyn	12	509	3100
Pilger	205	249	1785
Prince Albert	250	285	1432
Qu'Appelle	91	176	2147
Regina	87	217	1884
Rosthern	220	320	1672
St. Walburg	276	489	2050
Saskatoon	195	330	1600
Scott	202	466	2164
Shaunavon	37	442	3010
Swift Current	80	405	2440
Waseca	246	509	2105
Whitewood	78	75	1973
Yellow Grass	49	189	1899

The equations for the eleven other months differ only in the substitution on the right-hand side of the successive trios of products:

−8,946.6	−7,925.3	−2,596.1	−238.7	−1,622.4	−2,716.0
44,812.4	26,956.8	5,733.0	−9,306.3	−18,332.8	−14,700.7
144,407.8	85,699.6	4,237.4	−47,323.0	−65,805.9	−51,748.9

−2,998.0	−2,587.6	−3,459.7	−6,117.0	−9,056.8
−13,990.5	−10,374.1	4,300.1	21,281.5	35,197.7
−46,667.7	−34,908.0	11,178.7	69,882.9	122,253.0

TABLE II

MEAN TEMPERATURE (°F.), 1917–1934, BY MONTHS

Station	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Alberta												
Alix	9.9	14.0	24.0	39.7	50.4	57.4	62.8	60.5	49.9	39.3	25.8	11.6
Bassano	14.4	17.4	2 .7	41.6	53.1	60.7	65.9	64.4	53.7	42.4	28.7	15.7
Calgary	17.3	20.6	26.7	39.6	50.3	57.2	62.8	60.8	51.2	41.3	29.2	17.4
Calmar	8.6	13.4	21.6	37.6	49.1	55.6	60.1	58.0	48.6	3 .1	24.7	10.6
Edmonton	8.8	14.2	22.7	39.5	51.2	57.6	62.2	59 7	49.9	39.4	25.1	10.6
Gleichen	12.2	16.4	25.2	39.7	50.7	58.3	64.1	61.5	50.	40.2	26.7	14.2
Harmattan	11.7	15.8	22.1	36.3	46.3	53.7	57.9	55.8	46.3	36.9	24.8	12.7
High River	17.8	19.4	26.1	37.5	47.5	54.1	60.0	57.7	48.7	39.7	29.2	17.4
Hillsdown	13.3	16.5	24.6	39.1	50.4	56.8	62.7	59.3	49.4	39.9	26.8	14.4
Lacombe	9.7	14.2	22.4	38.2	49.1	56.3	61.5	59.1	49.2	39.0	25.2	11.0
Lethbridge	19.4	21.1	28.6	40.8	50.9	58.7	64.5	6 .4	52.2	43.1	31.2	19.6
Lundbreck	16.5	17.9	24.4	35.4	44.6	52.0	57.9	55.8	46.8	38.8	28.0	17.2
Macleod	20.6	22.3	30.2	41.8	52.4	60.2	66.4	64.1	53.8	45.0	32.2	20.7
Medicine Hat	16.4	19.7	29.9	44.8	56.7	64.9	70.9	67.3	55.7	45.1	31.2	17.8
Olds	13.7	16.7	23.4	38.1	47.8	51.8	59.7	57.7	48.0	38.9	26.2	14.6
Pekisko	17.5	19.1	24.1	34.2	43.1	50.1	55.7	53.9	45.3	38.2	28.2	17.1
Perbeck	10.8	14.6	23.1	38.8	50.1	57.2	63.2	60.1	49.5	38.8	25.1	11.9
Ranfurly	4.7	9.4	18.9	37.9	51.1	57.7	62.7	60.3	49.8	37.8	22.9	7.1
Strathmore	13.1	15.9	24.6	38.9	49.8	57.3	62.9	59.8	49.9	39.4	26.8	14.0
Saskatchewan												
Anglia	4.5	8.2	19.6	38.0	51.4	60.1	65.1	62.4	51.1	38.4	23.5	7.7
Battleford	2.7	7.9	19.3	38.6	52.8	60.6	65.3	63.0	52.4	39.8	23.6	6.4
Chaplin	7.3	10.9	23.2	38.8	51.9	61.4	66.3	63.5	52.6	39.1	24.7	9.4
Fort Qu'Appelle	2.3	7.6	19.6	38.4	51.4	60.6	66.1	64.2	53.1	39.9	23.2	7.1
Indian Head	2.3	7.5	18.8	36.8	50.6	60.1	65.2	62.6	51.5	38.4	22.3	6.7
Kamsack	− 2.6	1.7	14.6	35.8	51.1	59.7	63.9	61.4	51.3	37.9	20.3	2.6
Klintonel	12.5	15.2	22.8	37.3	48.6	57.1	63.2	60.9	49.9	39.2	26.4	14.4
Melfort	− 1.4	4.0	15.5	35.2	50.3	59.2	63.5	60.5	50.3	37.0	19.5	5.2
Moose Jaw	8.4	12.4	23.9	40.2	53.4	62.6	68.0	65.3	54.3	41.5	26.6	11.8
Muenster	− 1.6	3.9	15.2	35.6	49.6	58.2	62.8	60.0	49.9	37.3	20.2	3.1
Nashlyn	8.4	12.8	23.0	39.8	51.1	59.8	66.6	63.8	51.6	40.2	24.5	10.1
Pilger	− 0.3	5.4	15.7	36.1	50.9	59.3	64.0	61.9	51.4	38.9	21.1	4.5
Prince Albert	0.4	6.1	16.8	36.9	51.7	59.9	64.9	61 8	51.4	39.4	21.8	6.2
Qu'Appelle	4.5	8.8	20.2	37.6	51.2	60.1	64.9	62.7	52.3	39.6	23.4	8.4
Regina	3.9	8.3	20.3	38.4	52.1	61.2	66.2	63.6	53.1	39.2	22.8	7.8
Rosthern	− 0.4	5.3	16.4	36.9	51.9	60.2	64.9	62.1	51.0	38.3	21.2	5.5
St. Walburg	− 2.4	2.8	14.7	34.5	9.1	56.5	61.2	58.3	47.7	36.1	19.3	1.8
Saskatoon	1.8	5.6	17.7	36.9	51.5	60.3	6 .4	62.4	51.4	38.9	22.6	6.9
Scott	2.1	5.6	16.8	36.5	49.9	58.0	63.3	60.7	50.0	37.8	22.1	5.7
Shaunavon	13.3	15.8	24.8	39.2	50.8	59.2	65.1	63.1	52.3	40.7	27.5	14.3
Swift Current	12.1	14.8	25.2	40.7	53.2	61.8	67.2	64.9	53.4	41.6	27.4	14.1
Waseca	1.8	6.5	17.1	36.5	0.1	57.4	62.4	59.8	49.1	36.9	21.0	5.5
Whitewood	2.7	6.7	18.4	36.4	49.9	58.7	63.5	60.9	50.7	38.	22.3	7.2
Yellow Grass	5.9	9.2	21.6	38.8	51.8	61.8	66.2	63.8	52.8	39.2	23.9	8.4
Mean	8.0	11.9	21.6	38.1	50.5	58.4	63.7	61.2	50.8	39.4	24.9	10.6
Standard deviation	6.51	5.63	4.13	2.10	2.29	2.94	2.87	2.72	2.17	1.92	3.13	4.81

Solution of the equations was effected by the inverse matrix method as systematized by Fisher (1, Sec. 29), the multipliers used in the computation of the unknowns and their standard errors, expressed as millionths, being:

	$c_{.1}$	$c_{.2}$	$c_{.3}$
$c_{1.}$	8.309592	−2.383167	0.929300
$c_{2.}$		1.638321	0.496299
$c_{3.}$			0.200340

The regression coefficients obtained, together with their respective standard errors, are assembled in Table III. With the exception of those for altitude in January and December, which are both less than twice their standard error, all are individually statistically significant.

TABLE III

PARTIAL REGRESSION COEFFICIENTS OF 18-YEAR AVERAGE (1917–1934) MONTHLY MEAN TEMPERATURE ON LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS

Month	Partial regression coefficient of temperature on		
	Latitude (°F. per 10′ N.)	Longitude (°F. per 10′ W.)	Altitude (°F. per 100 ft.)
January	−.56 ± .05	+.25 ± .02	−.13 ± .08
February	−.47 ± .05	+.23 ± .02	−.16 ± .08
March	−.50 ± .04	+.21 ± .02	−.36 ± .06
April	−.31 ± .03	+.13 ± .01	−.44 ± .05
May	−.24 ± .03	+.09 ± .02	−.51 ± .05
June	−.31 ± .04	+.06 ± .02	−.56 ± .06
July	−.36 ± .05	+.08 ± .02	−.56 ± .08
August	−.35 ± .04	+.07 ± .02	−.51 ± .06
September	−.29 ± .03	+.06 ± .01	−.42 ± .05
October	−.29 ± .03	+.10 ± .01	−.31 ± .05
November	−.37 ± .03	+.15 ± .02	−.22 ± .05
December	−.46 ± .05	+.19 ± .02	−.14 ± .08

The three series of coefficients present certain features of interest, for the relation of mean temperature to each of the three variables, latitude, longitude and altitude, changes in a marked but generally regular manner with the progress of the year. Each series has an independent seasonal trend, which is simplest in the case of altitude and most complex in the case of latitude. The coefficients for altitude increase steadily in magnitude from a minimum of −0.13° F. per 100 ft. in January to a maximum of −0.56° in June, then progressively decline to −0.14° in December. This is in agreement with the observation of Kincer (4) that the decrease in air temperature with altitude is greater in summer than in winter. On the other hand, the effect of longitude is most pronounced in winter. In January the mean temperature increases from east to west by an average amount of 0.25° F. for each 10′ longitude, after allowance is made for the effects of latitude and altitude. This effect diminishes rapidly in spring, however, and continues at a low level with little variation from May to September. The influence of latitude is likewise most pronounced in winter, being at its maximum in January when, after allowing for differences in longitude and altitude, there is an average decrease of 0.56° per 10′ N. In this case, however, the series of monthly coefficients exhibits two minima, one in the spring and the other in the autumn.

Fig. 2 shows the situation in graphical form. The points in this diagram represent the actual values of the regression coefficients listed in Table III, whilst the continuous curves show the course of harmonic equations fitted

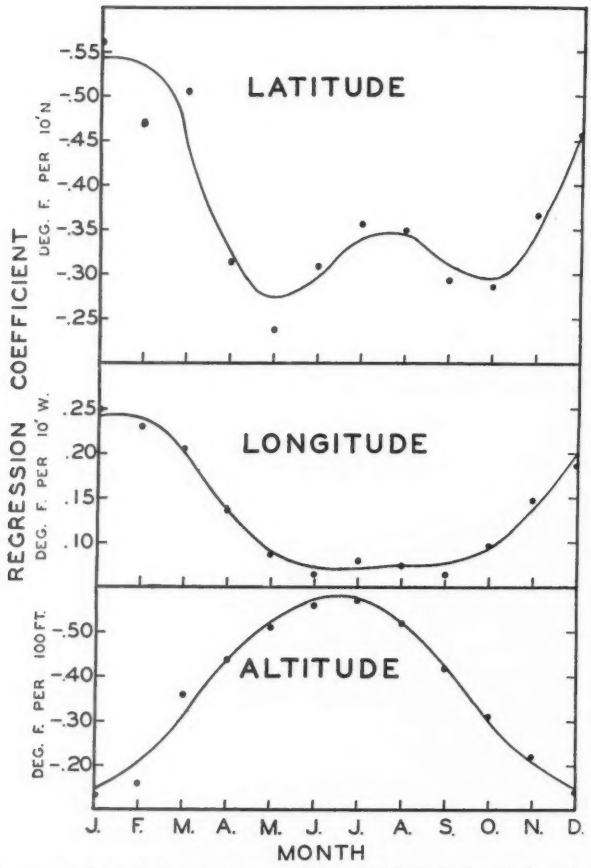

FIG. 2. *Seasonal trend of temperature gradients with latitude, longitude and altitude.*

to each series of 12 monthly coefficients by the method of Least Squares. The monthly regression coefficients of temperature on altitude follow very closely the simple harmonic equation

$$b_m = -0.36 + 0.22 \sin (\theta + 105°10')$$

where b_m is the regression coefficient for any specified month and $\theta = \frac{2\pi m}{12}$, m taking the values 0,1,2, ...,11 for the successive months of the year. A simple harmonic likewise accounts for most of the variation in the longitude coefficients, but in this case a statistically significant improvement results from the addition of a second harmonic term, giving

$$b'_m = 0.14 + 0.09 \sin (\theta + 76°10') + 0.02 \sin (2\theta + 57°55')$$

The latitude sequence is however represented only moderately well by

$$b''_m = -0.38 + 0.10 \sin (\theta + 261°50') + 0.08 \sin (2\theta + 241°40')$$

and the addition of the term in 3θ results in no significant improvement.

Table IV gives for each month the multiple correlation coefficient R between mean temperature and latitude, longitude and altitude, and the residual standard deviation s of the actual 18-year averages from the graduated values.

The correlation coefficients are all quite high, indicating that the linear regression equations have accounted for the major part of the recorded temperature differences between stations. When it is recalled that the residual standard deviation includes, as well as observational errors, any systematic non-linear deviations, any irregularities remaining in the station averages due to the small number of years included, and any effects arising from local topography or the exposure of individual stations, the results shown in Table IV cannot be regarded as wholly unsatisfactory. In some instances indeed it may well be that the graduated temperatures approximate more closely to the true values for the adjacent territory than do those recorded at the individual stations. It may be concluded therefore that Table III and Fig. 2 provide a fairly comprehensive numerical description of the temperature gradients characteristic of this region at different periods of the year.

TABLE IV

MULTIPLE CORRELATION COEFFICIENT (R) BETWEEN MEAN TEMPERATURE AND LATITUDE, LONGITUDE AND ALTITUDE, AND RESIDUAL STANDARD DEVIATION (s), BY MONTHS

Month	R	s, °F.
Jan.	0.96	1.9
Feb.	.96	1.7
Mar.	.95	1.3
Apr.	.87	1.1
May	.86	1.2
June	.91	1.3
July	.88	1.4
Aug.	.89	1.3
Sept.	.89	1.0
Oct.	.83	1.1
Nov.	.93	1.2
Dec.	.94	1.7

Results with Observations for Individual Years

The remarks of the preceding section apply of course to the 18-year averages of monthly mean temperature. In the means for any month of a single year, additional deviations from the regression equation may arise from two sources: (i) over the region as a whole, the temperature of the month in question may be above or below the climatological average; (ii) local irregularities, which tend to nullify each other in the averages of a number of years, will be more pronounced.

Annual differences of type (i), in so far as they affect all stations equally, need not increase the residual standard deviation, since they require only an adjustment in the constant term of the regression equation. Irregular local variations, on the other hand, will of course result in increased discrepancies between the observed and graduated values. In order to determine the extent of such effects in practice, the two extreme winter and summer months January and July were selected, and the total variance of the 18 × 43 annual monthly means for each was partitioned (1, Chap. VII) into components

due to (i) differences between the 18-year averages of the 43 individual stations; (ii) differences between the 43-station averages of the 18 individual years; and (iii) residual irregular local variation. These computations gave the mean square deviations shown in Table V.

TABLE V

ANALYSIS OF VARIANCE OF MONTHLY MEAN TEMPERATURE

Source of variation	Degrees of freedom	Mean square	
		January	July
Between station averages	42	785.38	147.72
Between year averages	17	2643.02	52.87
Residual	714	4.80	1.65

The January mean square between years is greatly in excess of the mean square residual, indicating a pronounced correlation between the annual variations in mean temperature of this month at the 43 stations. A similar tendency is to be noted in the results for July, but in this case the degree of intra-annual correlation indicated is appreciably lower.

TABLE VI

LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS, AND MONTHLY MEAN TEMPERATURES FOR THE YEAR 1935

Station	Lat. N. of 49°, min.	Long. W. of 103°, min.	Height above sea level, ft.	Monthly mean temp. 1935, °F.			
				Jan.	Apr.	July	Oct.
Beechy	110	266	2180	− 5.6	34.4	67.4	38.7
Biggar	183	299	2154	− 9.1	34.2	69.3	39.8
Caron	88	173	1841	− 2.5	37.7	68.8	38.4
Chaplin	88	220	2202	− 3.0	36.8	69.9	38.2
Davidson	136	179	2030	− 7.7	34.8	67.6	36.3
Dundurn	168	210	1737	− 8.3	35.2	69.0	38.7
Ft. Qu'Appelle	107	48	1600	− 4.6	36.8	67.0	37.9
Francis	67	50	1977	− 6.0	33.9	68.1	38.5
Gravelbourg	52	213	2297	− 3.8	36.3	68.9	38.4
Harris	164	273	1896	−10.4	33.9	68.1	41.9
Humboldt	192	129	1865	−11.6	31.9	68.0	38.3
Indian Head	88	40	1924	− 6.0	35.6	68.9	38.4
Lestock	137	57	2219	− 7.1	32.8	66.7	37.1
Lumsden	99	115	1620	− 5.0	37.7	68.8	39.8
Maskakee Springs	199	161	1787	−12.8	33.3	69.9	37.9
Moose Jaw	81	155	1860	− 1.1	37.7	70.4	39.9
Nokomis	150	120	1718	− 7.8	35.6	68.3	36.8
Outlook	148	245	1774	− 7.4	34.9	70.5	39.6
Pennant	92	314	2346	− 1.6	35.2	69.0	43.1
Qu'Appelle	91	56	2147	− 4.5	34.6	67.4	37.9
Regina	87	97	1884	− 5.8	36.9	68.7	38.5
Saskatoon	195	210	1600	−10.1	34.0	68.6	37.9
Saskatoon Univ.	188	218	1690	−10.0	34.5	69.2	38.6
Strasbourg	125	117	1799	− 6.8	35.7	67.3	39.3
Swift Current	80	285	2440	+ 2.3	36.4	68.4	40.4
Tugaske	111	196	1986	− 6.1	35.1	68.4	38.6
Yellow Grass	49	69	1899	− 2.6	35.3	69.2	38.8

When the residual variance in Table V, ascribable to local variation within years, was added to the residual variance of the 18-year station averages from the regression equation given in the preceding Table IV, standard deviations of 2.9° F. for January and 1.9° for July were obtained. These may be regarded as representative of the closeness of graduation to be expected on the average from the regression coefficients of Table III (after adjustment of the constant term), in individual years. The agreement thus indicated between the observed and graduated values can hardly be regarded as entirely satisfactory.

More recently, additional meteorological stations have been established, and records for the year 1935 are available for 27 stations situated within or just beyond the borders of the area bounded by the 50th and 52nd parallels and the 104th and 108th meridians. The mean temperatures for the months of January, April, July and October of that year at each of these points were accordingly tabulated, and are shown in Table VI, together with the latitude, longitude and altitude of the individual stations.

The Normal Equations to determine the regression coefficients of January mean temperature on latitude, longitude and altitude respectively were:

$$\begin{aligned} 54{,}264\ b_1 + 28{,}121\ b_2 - 116{,}241\ b_3 &= -3{,}496.1 \\ 28{,}121\ b_1 + 184{,}263\ b_2 + 180{,}373\ b_3 &= 168.5 \\ -116{,}241\ b_1 + 180{,}373\ b_2 + 1{,}432{,}561\ b_3 &= 10{,}371.2 \end{aligned}$$

whilst the corresponding values of the right-hand side for the other three months were:

−1093.2	25.9	−207.6
− 129.9	767.3	1721.5
− 707.6	−670.3	2146.2

Proceeding as before, the matrix of multipliers was found to be, in millionths

	$c_{.1}$	$c_{.2}$	$c_{.3}$
$c_{1.}$	29.83665	−7.89664	3.41527
$c_{2.}$		8.27989	−1.68326
$c_{3.}$			1.18711

giving the regression coefficients and standard errors shown in Table VII.

TABLE VII

PARTIAL REGRESSION COEFFICIENTS OF MEAN MONTHLY TEMPERATURE (1935) ON LATITUDE, LONGITUDE AND ALTITUDE OF METEOROLOGICAL STATIONS

Month	Partial regression coefficient of temperature on		
	Latitude (°F. per 10′ N.)	Longitude (°F. per 10′ W.)	Altitude (°F. per 100 ft.)
January	−.70 ± .09	.12 ± .05	.01 ± .17
April	−.34 ± .05	.09 ± .03	−.44 ± .10
July	−.08 ± .05	.07 ± .03	−.20 ± .10
August	−.12 ± .06	.12 ± .03	−.11 ± .13

These coefficients are of course affected by any circumstances peculiar to the particular year and locality. Nevertheless on the whole their seasonal trend resembles that of the series previously computed for the 43 more widespread 18-year stations, although there are some discrepancies. The values of R, the coefficient of multiple correlation between mean temperature and the three co-ordinates of position, and of s, the residual standard deviation, were found to be as follows for the four months:

Month	R	s, °F.
January	0.90	1.6
April	.82	0.9
July	.52	0.9
October	.64	1.2

The correlation coefficients, although all statistically significant, are lower than those listed in Table IV, and the standard errors of the regression coefficients are relatively high (Table V). This is not surprising, since in the smaller area now under consideration irregular local effects would be expected to constitute an increased proportion of the total variance in temperature. On the other hand, the residual standard deviations for the four months are now appreciably lower than those deduced on page 25. Some addition to the number of stations is still desirable to reduce the standard deviation of the coefficients, the 27 listed in Table VI being distributed over an area of approximately 25,000 square miles. If this was effected however, it would seem that a reasonably accurate linear graduation of the air temperature gradients in this district, even in individual years, would be possible.

References

1. FISHER, R. A. Statistical methods for research workers. 5th ed. Oliver and Boyd, London. 1934.

2. HOPKINS, J. W. Agricultural meteorology: Some characteristics of precipitation in Saskatchewan and Alberta. Can. J. Research, C, 14 : 319-346. 1936.

3. IRWIN, J. O. Crop forecasting and the use of meteorological data in its improvement. Conference of Empire Meteorologists, 1929, Agricultural Section, II : 220-276. H.M. Stationery Office, London. 1929.

4. KINCER, J. B. Climate: Temperature, sunshine and wind. (*In* U.S. Dept. Agr. Atlas of American Agriculture). U.S. Govt. Printing Office, Washington, 1928.

5. METEOROLOGICAL SERVICE OF CANADA, DEPT. OF TRANSPORT. Monthly record of meteorological observations in Canada and Newfoundland. Toronto.

6. SCHUMANN, T. E. W. Interpolation of monthly rainfall data. Quarterly J. Roy. Met. Soc. 62 : 435-446. 1936.

THE EFFECT OF PHOSPHATE DEFICIENCIES ON INFECTION OF WHEAT BY *FUSARIUM CULMORUM*[1]

By F. J. Greaney[2]

Abstract

The influence of phosphate deficiencies on infection of wheat by *Fusarium culmorum* (W. G. Sm.) Sacc. was studied. Marquis wheat was grown in pot cultures of quartz sand with different types of manuring, including a fully manured control, and four series having deficiencies of phosphate. One-half of the pots were inoculated with *F. culmorum* and sown with inoculated seed, the remainder served as uninoculated controls. The plants were grown for 36 days. The experimental data were treated by the analysis of variance method.

Under the conditions of the experiment, deficiencies in phosphate did not significantly increase or decrease the susceptibility of wheat plants to root rot caused by *F. culmorum*. On the other hand, deficiencies in phosphate significantly reduced root development and total dry weight of the plants. The results suggest that the effect of phosphatic fertilizers is much more important on plant growth and yield than on the severity of infection by *F. culmorum*.

Introduction

For many years considerable attention has been paid to mineral nutrition as a factor affecting susceptibility to disease in the higher plants. This subject has been investigated in a number of fungus diseases of wheat, barley, and other small grain crops. In the literature, however, references to the influence of phosphorus on disease development are often of a casual nature, made in the course of investigations with other plant nutrients. It may be well, therefore, before reporting the results of the present study which deals particularly with phosphorus, to mention some of the results obtained by other workers on the effect of mineral nutrition in relation to disease in plants.

One of the earliest important contributions to this subject is by Spinks (16). According to him, heavy nitrogenous manuring increases the susceptibility of wheat and barley to their particular rusts and mildews, whereas potash acts in a contrary manner. He found that the effect of phosphorus upon susceptibility to disease in cereal plants is not always the same: in some cases it decreases susceptibility and in others it does not. The results of more recent work by Schaffnit and Volk (14), Gassner and Hassebrauk (4, 5), Eglits (2), and by some others, are essentially similar to those of Spinks.

The effect of fertilizers on the development of obligate parasites, such as the rusts of wheat, have been studied by several investigators. For instance, Biffen (1) and Voelcker (20) found that heavy applications of nitrogenous fertilizers increased the susceptibility of wheat to *Puccinia glumarum*. Spinks (16) and Voelcker (20) concluded that potassium and phosphorus salts increased the resistance of wheat to stripe rust but did not counteract the effect of large amounts of nitrogen. Vavilov (19) concluded that the apparent increased susceptibility of wheat to *Puccinia triticina*, when grown in soil

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fertilized with nitrogen, was due to increased development of leaf surface rather than to any change of real resistance. Stakman (17) and Stakman and Aamodt (18) found that excessive amounts of phosphorus salts had no direct effect on the resistance of wheat to *Puccinia graminis*, and concluded that any decrease in the amount of this rust due to phosphatic fertilizers was brought about by hastening the maturity of the host plant. Similar results with *Puccinia triticina* were reported by Greaney and Machacek (7). Most of these results indicate that nitrogen and potash influence the susceptibility of wheat to disease. The evidence, however, in regard to any clear-cut effect of phosphorus on resistance in wheat, even to such obligate parasites as the rust fungi, is not very convincing.

In regard to facultative parasites that attack crop plants, Neal (13), in 1927, observed that increasing the phosphate content of the soil did not decrease the tendency of cotton plants to become infected by *Fusarium vasinfectum* Atk. The work of McRae and Shaw (12) on the influence of manures on the wilt disease of Indian Pulse (*Cajanus indicus* Spreng.) indicated that manuring with superphosphate increased attack by *Fusarium vasinfectum*. They found that increasing the concentration of phosphate increases the growth of the fungus up to a certain point, after which higher concentrations check growth. McRae and Shaw (12) reported that, in sand culture experiments with cotton seedlings, Kulkarni and Mundkur found that high concentrations of potassium phosphate predispose plants to attack by *F. vasinfectum*.

The influence of mineral nutrition upon susceptibility to disease in plants has been studied for a number of fungi, with results that are in many cases indefinite. The records of these investigations do not give any clear proof that phosphorus directly affects the natural resistance to disease that wheat plants possess. It was therefore decided to grow numbers of wheat plants in sand culture under varying conditions of nutrition and to observe any differences in their susceptibility to the attacks of root rot caused by *Fusarium culmorum*. This paper presents the results of greenhouse studies to determine the effect of deficiencies in phosphate on infection of young wheat plants by *Fusarium culmorum* (W. G. Sm.) Sacc.

Experimental Methods

It was expected from the results of previous studies (9, 10) with pot cultures in quartz sand that the effects of deficiencies of phosphorus on the resistance of wheat seedlings to *Fusarium culmorum* would be of small magnitude, and would require data of a high order of precision for their establishment. The technique of growing plants in sand culture described by Gregory and Crowther (9), in their studies on the differential response in yield of barley varieties to manurial deficiencies, seemed well adapted for the investigation of the problem in question.

A preliminary sand-culture experiment with wheat grown under various phosphate manurings was carried out in 1932. Four different concentrations

of phosphorus, including a complete manure, two deficiency series, and a series receiving an excess of phosphorus, were employed. The complete manure provided nitrogen, potash, and phosphorus in the ratio of 3 : 2 : 1. In each deficiency series nitrogen and potash were provided in the same concentration as in the control series with complete manure, while phosphorus was lacking in one and present in $\frac{1}{5}$ of the complete amount in the other. The fourth manurial series received twice the amount of phosphorus supplied in the complete fertilizer. An unmanured control was included in the experiment. This preliminary test with about 50 inoculated and 50 uninoculated plants in each manurial series was made at two different times. The resulting data were treated by the analysis of variance method.

Evidence was thereby produced, which indicated that the pathogenicity of *Fusarium culmorum* to wheat might be influenced by the amount of phosphate given. The results of the test suggested a wider range of manuring, and led to a more efficient method of artificially inducing attacks with *F. culmorum* in sand culture.

In view of these results it was decided to repeat the experiment using a greater range of phosphate deficiencies. This enlarged greenhouse experiment was conducted at Winnipeg in 1933, repeated at Winnipeg in 1934, at Rothamsted Experimental Station, Harpenden, England, in 1935, and again at Winnipeg in 1936. During the course of each trial, uniform conditions of light, moisture, and temperature prevailed. At Winnipeg the temperature range was from 22° C. to 25° C.; while the range at Rothamsted was from 20° C. to 26° C. To minimize place effect the pots of each trial were completely randomized on a large bench in the centre of the greenhouse.

The complete experiment involved 60 pots. The pots were of white glazed earthenware holding 15 pounds of dry white quartz sand. Before use, the sand was washed three times in tap water, rinsed twice in distilled water, and sterilized under steam pressure. The manures were added in solution and the pots of sand brought up to uniform moisture condition by the addition of distilled water. The pots were then planted with seed of Marquis wheat. The water content was maintained at a uniform level by adding distilled water at two-day intervals to bring the pots up to their original weight. Waterlogging in the pots was avoided by allowing percolation. The percolated water was collected and subsequently returned to the pots. The manuring scheme employed in the experiment is given in Table I.

TABLE I
MANURIAL TREATMENTS
(Amounts in grams per pot)

Series / Constituent	Complete manure	Phosphate-deficient series				Unmanured control
	A	B	C	D	E	F
N	0.75	0.75	0.75	0.75	0.75	0.0
K_2O	0.50	0.50	0.50	0.50	0.50	0.0
P_2O_5	0.25	0.05	0.025	0.0125	0.0	0.0

The complete manure provided nitrogen (N), potash (K_2O), and phosphoric acid (P_2O_5) in the ratio of 3 : 2 : 1. In each deficiency series the two constituents not in deficiency were supplied in the same concentration as in the series with complete manure, while phosphorus was present in 0, $\frac{1}{20}$, $\frac{1}{10}$, and $\frac{1}{5}$ of the complete amount. This gave a range of grades of deficiency in phosphorus in the presence of adequate amounts of the other constituents. Nitrogen was added as $NaNO_3$, phosphate as Na_2HPO_4, $12H_2O$, and the potash as K_2SO_4. Calcium (0.19 gm. $CaCl_2$), magnesium (0.63 gm. Mg_2SO_4, 7 H_2O), and a trace of iron ($FeCl_3$) were added to each pot. The solutions were brought to an initial pH of 5.8 by adding sulphuric acid. Resulting variations in the amount of sodium were corrected by adding Na_2SO_4, 10 H_2O. On the basis of a 19% water content of the sand, the concentration of sodium was far below that necessary to produce the slightest toxic effect (11). The only other ion that varied was SO_4. It has been shown to have a very small effect on plant growth in sand culture (8).

The seed used was Marquis wheat, selected by hand for uniformity in size and color. It was surface-sterilized by rinsing in 75% ethyl alcohol, immersing for three minutes in 0.1% $HgCl_2$ solution, rinsing in alcohol, and then washing in sterile water. Seed treated in this manner gave a germination of 99% in blotting-paper and sand tests.

Before sowing, one-half of the surface-sterilized seed was inoculated by dipping it in a suspension of spores and mycelial fragments of *Fusarium culmorum*. The control seed was dipped in sterile water. Both lots were sown immediately after treatment.

The particular fungus used in this investigation was a strain of *Fusarium culmorum* (W. G. Sm.) Sacc. that was originally isolated in 1930 from a rotted crown of Marquis wheat. Previous tests (6) had demonstrated that this strain was definitely pathogenic to wheat.

In each manurial series there were 10 pots, five sown with inoculated seed and five with uninoculated seed to serve as control. Twenty seeds were sown in each pot. By means of a small sterile glass rod, holes were made to a uniform depth of one inch below the surface of the sand. A single seed was placed in each hole. About 5 cc. of a water suspension of spores of *F. culmorum* was poured over each inoculated seed before it was finally covered with sand. The same quantity of sterile water was poured over each uninoculated seed. The seeds were covered by lightly packing the surface layer of sand.

Germination was usually complete 10 days after sowing, at which time the plants in each pot were reduced to a uniform number. In three of the four trials, the total number of post-emergence plants per inoculated and uninoculated series of each manurial type was about 95.

At the end of the experimental period (36 days), non-emerged plants as well as the young seedlings were lifted from the pots, washed free of sand, examined individually, and the extent of injury due to pre-emergence blight, seedling blight, or root rot was recorded. The classes and numerical ratings

used to record the intensity of disease infection on individual plants, and the method of computing the disease rating that was used to express the extent of the disease on the plants in each series of pots, are given in Table II.

TABLE II

CLASSES, NUMERICAL RATINGS, AND DISEASE RATING USED TO RECORD THE DEGREE OF INFECTION BY *Fusarium culmorum* ON WHEAT PLANTS

Class	Degree of infection on individual plants	Numerical rating
1	No infection	0
2	Small, scattered necrotic lesions on sheath, sub-crown internode, or roots	1
3	Distinct lesions on basal parts, particularly on sub-crown internode and roots	2
4	Large necrotic lesions on crown, sub-crown internode and roots, with loss of plant vigor	3
5	Severe rotting of basal parts; plants chlorotic, often stunted or wilted; some culms dead	4
6	Plant destroyed after germination but before emergence. Dead plant	5

$$\textit{Disease rating} = \frac{\textit{Sum of numerical ratings} \times 100}{\textit{Number of plants at 36 days} \times 5}$$

After the disease data had been secured, the plants were air-dried and the total dry weight per inoculated and uninoculated set of each manurial series was recorded.

Plant emergence, disease, and yield data were analyzed according to the procedure described by Fisher (3) as the analysis of variance. To estimate the odds of significance, however, the direct ratio of the variances, the F value of Snedecor (15), was used.

Experimental Results

The results of the preliminary experiment in 1932 (Table III) show the effects of different manurings with phosphate on infection by *Fusarium culmorum* and on the growth of young wheat plants. To economize space, the complete analysis of variance for disease-infection rating and total dry weight of plants is not given. The results of these analyses, however, established that, for the amount of disease and total dry weight, the effects of seed inoculation with *F. culmorum* were very great. In this experiment as a whole, the differences in disease infection for manurial treatments were not statistically significant; whereas the differences observed in plant growth, due to different phosphate manures, were very significant.

The evidence presented in Table III shows that there is a tendency, although the differences are not significant statistically, for decreasing concentrations of phosphate to increase infection by *F. culmorum*. Under the conditions of the experiment, an excess of phosphate still further increased infection by this fungus. Decreasing concentrations of phosphate decreased root development and general plant growth.

TABLE III

INFLUENCE OF DIFFERENT PHOSPHATE MANURES ON INFECTION OF WHEAT BY *Fusarium culmorum*, AND ON TOTAL DRY WEIGHT OF PLANTS
(Average results of two preliminary trials)

Manurial series	Manuring scheme, gm. per pot			Degree of infection (disease rating)		Total dry weight,* gm.	
				Seed treatment			
	N	K_2O	P_2O_5	Inoculated	Control	Inoculated	Control
Complete	0.75	0.5	0.25	41.8	8.0	6.4	8.2
P-deficient	0.75	0.5	0.05	47.6	7.2	6.1	7.8
P-deficient	0.75	0.5	0.0	60.2	8.4	4.2	6.6
No fertilizer	0.0	0.0	0.0	60.1	14.2	3.6	5.4
P-excess	0.75	0.5	0.5	64.7	5.7	3.6	6.2
Mean of seed treatments				54.9	8.7	4.8	6.8
S.E. of means of seed treatments				±2.51		±0.22	

**Standard error of manurial treatments for total dry weight = ±0.16.*

The complete data of the enlarged experiment with six manurial series—a fully manured control, four deficiency series, and a complete starvation series— are given in Table IV. This table presents the data recorded on percentage of plants emerged, percentage of plants diseased, intensity of infection by *F. culmorum*, and total dry weight of plants per series of five pots with inoculated and with uninoculated seed. The figures are the means of each series.

The data of the four trials were treated by the analysis of variance method (Table V). The significance of the results were assessed by the F test in which the variance due to any known cause is compared directly with the variance due to error.

The results of the analyses in Table V show that the effects of seed inoculation with *F. culmorum* are very great, as would be expected. In every case the variances for seed treatments greatly exceed the error variances. Thus the results establish the efficiency of the method used to induce positive attacks with *F. culmorum*.

The efficiency of the method being established, a more detailed examination was made of the effects of deficiencies of phosphate on disease development and plant growth. This examination of the data shows that, owing to the four stages of deficiency of phosphate employed, the differences in number of plants emerged after 10 days, and in number of plants diseased, and degree of infection by *F. culmorum* after 36 days, are not significant. In Table V the variance for manurial treatments in the case of plants emerged, plants diseased, and disease rating, is not as great as, or significantly greater than, the variance due to error. This establishes, therefore, that the differences observed between manurial treatments might easily have arisen from chance alone.

TABLE IV

PERCENTAGE OF PLANTS EMERGED, PERCENTAGE OF PLANTS DISEASED, DEGREE OF INFECTION BY *Fusarium culmorum*, AND TOTAL DRY WEIGHT OF WHEAT PLANTS IN FOUR TRIALS WITH DIFFERENT PHOSPHATE MANURES

Trial	Seed treatment	Complete fertilizer (N.P.K.)	Phosphate-deficient series				Unmanured control
			$\frac{1}{5}$ P	$\frac{1}{10}$ P	$\frac{1}{20}$ P	0 P	
Percentage of plants emerged							
Winnipeg, 1933	Inoculated	100	97	99	99	100	100
	Uninoculated	100	99	100	99	98	100
Winnipeg, 1934	Inoculated	99	95	96	100	97	93
	Uninoculated	96	100	100	100	98	100
Rothamsted, 1935	Inoculated	100	98	98	100	98	98
	Uninoculated	100	100	100	100	100	100
Winnipeg, 1936	Inoculated	65	75	85	81	70	76
	Uninoculated	94	97	95	95	94	94
Percentage of plants diseased							
Winnipeg, 1933	Inoculated	100	100	100	100	100	100
	Uninoculated	20	28	29	22	23	43
Winnipeg, 1934	Inoculated	100	100	100	98	100	100
	Uninoculated	39	30	34	25	11	29
Rothamsted, 1935	Inoculated	67	78	82	80	93	85
	Uninoculated	13	12	13	8	12	18
Winnipeg, 1936	Inoculated	100	100	100	100	100	100
	Uninoculated	13	16	21	17	43	57
Degree of infection (Disease rating)							
Winnipeg, 1933	Inoculated	62.5	68.0	70.2	70.3	54.9	53.2
	Uninoculated	4.8	6.6	6.2	6.8	6.2	10.0
Winnipeg, 1934	Inoculated	50.7	50.7	41.3	33.4	43.4	34.6
	Uninoculated	12.0	8.4	9.0	5.0	5.6	6.2
Rothamsted, 1935	Inoculated	13.3	15.6	16.9	20.0	25.1	24.4
	Uninoculated	3.4	3.0	3.3	1.7	2.3	3.7
Winnipeg, 1936	Inoculated	47.6	62.7	66.4	63.6	62.6	72.3
	Uninoculated	3.0	3.6	4.4	3.4	10.3	24.8
Dry weight of plants (grams)							
Winnipeg, 1933	Inoculated	23.4	20.3	22.5	16.5	19.0	11.2
	Uninoculated	24.5	24.3	27.1	22.7	19.5	14.8
Winnipeg, 1934	Inoculated	15.4	14.5	17.8	14.5	11.0	10.0
	Uninoculated	17.6	18.7	17.9	19.3	15.2	12.1
Rothamsted, 1935	Inoculated	14.0	15.2	14.0	14.8	13.6	8.8
	Uninoculated	17.8	16.2	14.1	15.4	14.3	9.4
Winnipeg, 1936	Inoculated	8.5	7.9	9.1	8.5	8.5	3.5
	Uninoculated	14.9	13.8	14.4	14.3	14.2	6.2

It is clear from Table V, however, that a significant difference for manurial treatments is obtained in the case of total dry weight of plants. The F value greatly exceeds the 5% point. This result indicates that a high degree of significance can be attached to the effect of phosphate deficiencies on plant

TABLE V

COMPLETE ANALYSIS OF VARIANCE FOR PERCENTAGE OF PLANTS EMERGED, PERCENTAGE OF PLANTS DISEASED, DISEASE RATING, AND TOTAL DRY WEIGHT OF PLANTS

Variance due to	Degrees of freedom	Sum of squares	Mean square	F	5% point
	Percentage of plants emerged				
Experiments	3	1,711.73	570.57		
Fertilizers	5	46.73	9.35	—	—
Seed treatments	1	408.33	408.33	13.31	4.12
Fertilizers × seed treatments	5	26.17	5.23		
Error	33	1,012.27	30.67		
Total	47	3,205.23			
	Percentage of plants diseased				
Experiments	3	2,944.23	981.41		
Fertilizers	5	1,081.94	216.38	2.04	2.48
Seed treatments	1	58,590.18	58,590.18	554.20	4.12
Fertilizers × seed treatments	5	825.94	165.18	1.56	2.48
Error	33	3,489.03	105.72		
Total	47	66,931.32			
	Disease rating				
Experiments	3	4,697.48	1,565.82		
Fertilizers	5	80.81	16.16	—	—
Seed treatments	1	19,602.08	19,602.08	146.10	4.12
Fertilizers × seed treatments	5	120.46	24.09		
Error	33	4,427.60	134.17		
Total	47	28,928.43			
	Total dry weight				
Experiments	3	637.49	212.49		
Fertilizers	5	333.44	66.69	20.90	2.48
Seed treatments	1	121.92	121.92	38.22	4.12
Fertilizers × seed treatments	5	6.25	1.25		
Error	33	105.20	3.19		
Total	47	1,204.30			

growth as expressed by total dry weight. In all cases the interactions in the experiment are not significant.

The results of the experiment, with the standard errors associated with the various factors studied, are summarized in Table VI.

TABLE VI

INFLUENCE OF DEFICIENCIES OF PHOSPHATE ON THE PATHOGENICITY OF *Fusarium culmorum* TO WHEAT SEEDLINGS, AND ON PLANT GROWTH
(Average of four trials)

Manurial series	Manuring scheme, gm. per pot			Percentage of plants emerged (after 10 days)		Percentage of plants diseased (after 36 days)	
				Seed treatment			
	N	K_2O	P_2O_5	Inoculated	Control	Inoculated	Control
Complete	0.75	0.50	0.25	91.0	97.5	91.8	21.2
P-deficient	0.75	0.50	0.05	91.2	99.0	94.5	21.5
P-deficient	0.75	0.50	0.025	94.5	98.8	95.5	24.2
P-deficient	0.75	0.50	0.0125	95.0	98.5	94.5	18.0
P-deficient	0.75	0.50	0.0	91.2	97.5	98.3	22.2
Control	0.0	0.0	0.0	91.8	98.5	96.2	44.2
Mean of seed treatments				92.4	98.3	95.1	26.9
S.E. of means of seed treatments				±1.13		±2.10	
				Degree of infection (Disease rating)		Total dry weight of plants, (gm)*	
Complete	0.75	0.50	0.25	43.5	5.8	15.3	18.7
P-deficient	0.75	0.50	0.05	49.2	5.4	14.5	18.2
P-deficient	0.75	0.50	0.025	48.7	5.7	15.8	18.4
P-deficient	0.75	0.50	0.0125	46.8	4.2	13.6	17.9
P-deficient	0.75	0.50	0.0	46.5	6.1	13.0	15.8
Control	0.0	0.0	0.0	46.1	11.2	8.4	10.6
Mean of seed treatments				46.8	6.4	13.4	16.6
S.E. of means of seed treatments				±2.36		±0.36	

**Standard error of manurial treatment for total dry weight = ±0.63.*

The evidence presented in Table VI establishes the fact that positive attacks were induced by inoculating the seed and sand with spores and mycelial fragments of *F. culmorum*. The inoculation reduced plant emergence 5.9%, increased the number of diseased plants 68.2%, and raised the disease rating from 6.4 to 46.8. In each manurial series plants from inoculated seed were much smaller than plants from uninoculated seed (Fig. 1, A). The difference between the mean total dry weight of inoculated plants and uninoculated plants is 3.2 gm. This value exceeds three times the standard error of the means for total dry weight of plants. It can therefore be assumed that the difference of 3.2 gm. indicates a real difference between inoculated and uninoculated plants.

Under the conditions of the experiment, increasing deficiencies of phosphate did not render young wheat plants more susceptible to *Fusarium culmorum*. On the other hand, evidence was obtained which indicated that high concentrations of phosphate tend to predispose plants to attack. This result is

similar to that found by Neal (13) and by McRae and Shaw (12) with *Fusarium vasinfectum*. The results herein reported show that deficiencies in phosphate reduced root development and decreased the vigor of wheat seedlings (Fig. 1,B).

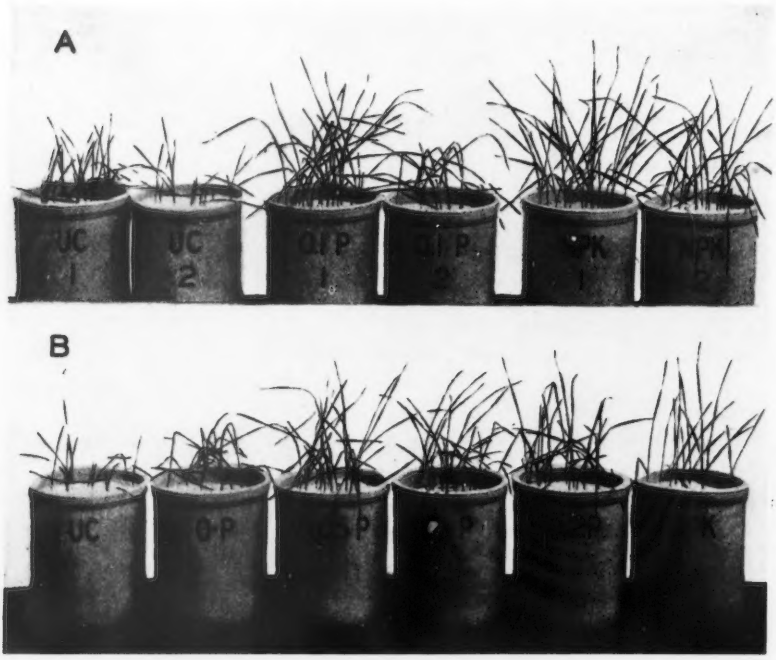

FIG. 1. *Influence of phosphate deficiencies on infection of wheat by Fusarium culmorum, and on plant growth. A. Effect of inoculation with F. culmorum. UC, unmanured control; NPK, complete manure; O.1-P, received $\frac{1}{10}$th the amount of phosphorus provided in NPK. Pot 1, uninoculated control; Pot 2, seed and sand inoculated with F. culmorum. B. Effect of deficiencies in phosphate on the growth of Marquis wheat at 15 days. UC, unmanured control; NPK, complete manure. In other series nitrogen (N) and potash (K) supplied in the same concentration as in the complete manure, while phosphorus (P) was given in the ratio of 0.0, 0.05, 0.1, and 0.2 to the complete amount.*

In the present experiments, the fungus *F. culmorum* attacked the roots of young wheat plants with equal vigor, regardless of the concentration of phosphoric acid employed; no statistically significant differences in their resistance to *F. culmorum* were established. The results gave no clear proof that phosphate directly affects the natural resistance of wheat plants to a root disease caused by *F. culmorum*, or affected in any way the parasitic vigor of the pathogen.

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References

1. BIFFEN, R. H. Studies in the inheritance of disease resistance. II. J. Agr. Sci. 5 : 421-429. 1912.
2. EGLITS, MAX. Die Empfänglichkeit des Winterroggens für *Puccinia dispersa* in Abhängigkeit von der Mineralfalzernährung. Ernähr. Pflanze. 30 : 167-180. 1934.
3. FISHER, R. A. Statistical methods for research workers. 319 pp. 5th ed. Oliver and Boyd, Edinburgh and London. 1934.
4. GASSNER, G. and HASSEBRAUK, K. Untersuchungen über die Beziehungen zwischen Mineralsolzernährung und Verhalten der Getreidepflanzen gegen Rost. Phytopath. Z. 3 : 535-617. 1931.
5. GASSNER, G. and HASSEBRAUK, K. Ueber die Beeinflussung der Rostanfälligkeit durch Eintauchen geimpfter Blatter in Losungen von Mineralsalzen und anderen Stoffen. Phytopath. Z. 5 : 323-342. 1933.
6. GREANEY, F. J. and MACHACEK, J. E. Studies on the control of root-rot diseases of cereals. I. Field methods with root-rot diseases. Sci. Agr. 15 : 228-240. 1934.
7. GREANEY, F. J. and MACHACEK, J. E. Effect of fertilizers on the incidence and severity of cereal diseases. In Progress Report of the Dominion Botanist, 1931-34, p. 33. Ottawa, 1935.
8. GREGORY, F. G. The differential effect of the ions of three salt solutions on the growth of potato plants in sand culture. Proc. Roy. Soc. (London), B, 102: 311-327. 1928.
9. GREGORY, F. G. and CROWTHER, FRANK. A physiological study of varietal differences in plants. I. A study of the comparative yields of barley varieties with different manurings. Ann. Botany, 42 : 757-770. 1928.
10. GREGORY, F. G. and CROWTHER, Frank. A physiological study of varietal differences in plants. II. Further evidence for the differential response in yield of barley varieties to manurial deficiencies. Ann. Botany, 45 : 579-592. 1931.
11. LIPMAN, C. B., DAVIS, A. R. and WEST, E. S. The tolerance of plants for NaCl. Soil Sci. 22 : 303-322. 1926.
12. MCRAE, W. and SHAW, F. J. F. Influence of manures on the wilt disease of *Cajanus Indicus* Spreng. and the isolation of types resistant to the disease. Imperial Council of Agr. Research, Sci. Monograph. No. 7. 1933.
13. NEAL, D. C. Cotton wilt. Ann. Missouri Botan. Garden, 14 : 359-424. 1927.
14. SCHAFFNIT, E. and VOLK, A. Ueber den Einfluss der Ernährung auf die Empfänglichk it der Pflanzen für Parasiten. Forsch. Gebiet. Pflanzenk. 3 : 1-45. 1927.
15. SNEDECOR, G. W. Calculation and interpretation of analysis of variance and co-variance. 96 pp. Collegiate Press, Inc., Ames, Iowa. 1934.
16. SPINKS, G. T. Factors affecting susceptibility to disease in plants. J. Agr. Sci. 5 : 231-247. 1913.
17. STAKMAN, E. C. A study in cereal rusts. Minn. Agr. Expt. Sta. Bull. 138. 1914.
18. STAKMAN, E. C. and AAMODT, O. S. The effect of fertilizers on the development of stem rust of wheat. J. Agr. Research, 27 : 341-379. 1924.
19. VAVILOV, N. I. Beiträge sur frage über die verschiedene Widerstandsfähigkeit der Getreide gegen Parasitische Pilze. In Trudy Selek. Stan. Moskov. Selskokhoz. Inst. 5 : 1-108. 1913.
20. VOELCKER, J. A. The Woburn pot-culture experiments, 1910-11-12. J. Roy. Agr. Soc. 5 : 314-338. 1912.

THE ORIGIN OF ABNORMAL RUST CHARACTERISTICS THROUGH THE INBREEDING OF PHYSIOLOGIC RACES OF *PUCCINIA GRAMINIS TRITICI*[1]

By Thorvaldur Johnson[2] and Margaret Newton[3]

Abstract

The inbreeding of physiologic races of *Puccinia graminis Tritici* by means of the selfing of certain selected strains for several successive generations has given rise to rust strains with various abnormal characteristics manifested not only in the uredial and telial stages but also in the pycnial and aecial stages. These abnormalities include: (i) Abnormal uredial color—grayish-brown, orange, white. (ii) A decrease in the vigor of sporulation, that is, a tendency to form uredia that fail to rupture the epidermis of the wheat plant. (iii) A decrease of pathogenic vigor in certain strains, as shown, for example, by a tendency towards an "x" type of infection in cultures descending from strains that appeared homozygous for the more vigorous "4" type of infection. Certain strains originating through selfing have also shown a greater sensitivity to high temperatures than strains collected in the field. Wheat varieties susceptible to such strains at ordinary greenhouse temperatures develop resistance towards them at temperatures above 80° F. whereas their reaction to cultures collected in the field remains almost unaffected. (iv) Loss of ability to produce aecia on the barberry. (v) The development of uredia and telia on the barberry by some strains that have, partially or entirely, lost the capacity to produce aecia.

The development of abnormal strains of rust is not an inevitable consequence of inbreeding, as many inbred strains show no abnormal characteristics. It is suggested that the abnormal characteristics are, in most cases, the result of recessive mutations that have taken place in the past history of the rust, the part played by the selfing being that of segregating and recombining the mutant factors in a homozygous state under which condition their effects are manifested in various types of abnormalities.

Introduction

For several years the writers have attempted to study the inheritance of pathogenicity and other characteristics of physiologic races of *Puccinia graminis Tritici* Erikss. & Henn. This study has been carried out by means of crosses or matings between the races, which, in turn, were followed by progeny studies involving the selfing of certain selected strains for several successive generations. Certain aspects of this work have been dealt with in previous papers (7, 10, 12, 13). In the course of these selfing studies, rust strains with various abnormal characteristics have appeared. The purpose of the present paper is to describe these abnormalities and, as far as possible, to explain their origin in the light of our present knowledge of the rust organism.

Before these abnormalities are considered it may be advisable to describe briefly the manner in which the selfing of physiologic races is carried out. In selfing a physiologic race, the race is first brought into pure culture by starting from a single urediospore, a single uredium, or a single aecial cup (aecium). The pure culture is then used to inoculate adult wheat plants on which teliospores subsequently develop. In order to ensure that no contamination by

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other races has taken place during this process it is essential to ascertain the purity of the race just prior to the formation of teliospores on the adult plants. When the teliospores have passed through their period of dormancy and have been induced to germinate they are used to inoculate barberries. After the appearance of the pycnial stage the pycniospore-containing nectar of numerous pustules is carefully intermixed, precautions being taken to prevent any accidental contamination by the pycnial nectar of other races. The resulting aecia are then used to inoculate wheat seedlings. With the appearance of the uredial stage on the wheat seedlings, the physiologic race has passed through its whole life cycle, for example, from an F_1 to an F_2 generation, without any intercrossing with other races. The physiologic race has therefore been selfed. Repeated selfings, generation after generation in the same line, amount to a process of inbreeding and may eventually result in genetically pure lines of the rust. Inbreeding for several successive generations, such as has been carried out in the laboratory, presumably occurs rarely, if ever, in nature.

One of the difficulties encountered in selfing studies is that of securing a population truly representative of the generation (F_2, F_3, etc.) that is to be studied—a matter which has already been discussed in some detail by the present writers (10, pp. 38–39). The method that has been adopted comprises a random selection of aecia, each aecium being used to initiate a uredial culture. As such cultures commonly contain but one physiologic race each, this method ensures that isolated phenotypes are being studied rather than mixtures of phenotypes, as frequently happens when all the aecia of a pustule are used to establish a culture.

The Effect of Inbreeding on the Uredial Stage

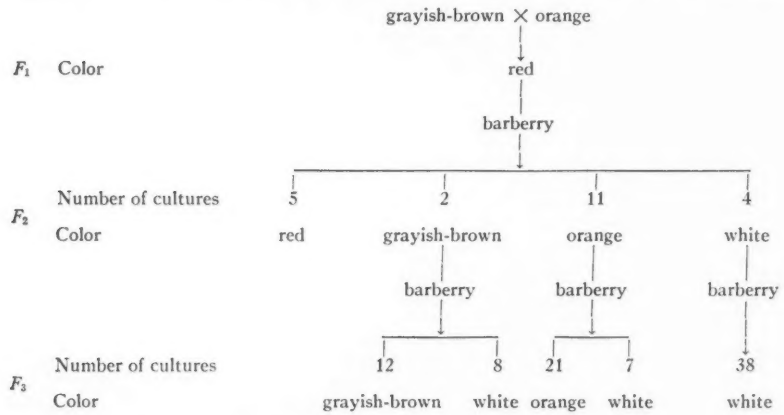
The Occurrence of Strains of Abnormal Uredial Color

Strains of abnormal uredial color are very rarely found in field collections of stem rust. Although field collections from various parts of Canada have been studied annually for 18 years, only one authentic record exists of the collection of uredia of abnormal color. As these uredia, which were grayish-brown in color, were collected on grasses in close proximity to an infected barberry plant, it is probable that they originated from infections caused by aeciospores. Although such strains are rarely found in nature, they have been known to originate in the greenhouse as mutants from races of normal uredial color. One such mutant with orange uredia has been reported by Newton and Johnson (9), and another with uredia designated as Mars Yellow has been described by Waterhouse (16). Nevertheless, cultures of stem rust, while they remain in the uredial stage, rarely give rise to such strains. When, however, barberries are infected with sporidia from teliospores collected in nature, some of the resulting aecia occasionally produce races of abnormal uredial color. The first record of a color variant originating in this manner—Race 36 (grayish-brown) of wheat stem rust—was described by Newton and Johnson (9). The origin, in a similar manner, of uredia of abnormal color has

also been recorded in other varieties of stem rust. Newton and Johnson (10, p. 34) obtained grayish-brown uredia from aecia on a barberry plant infected with a field collection of teliospores of *Puccinia graminis Secalis* Erikss. and Henn. In oat stem rust, color variants with orange uredia have been obtained by Gordon (4, p. 189) from a barberry that had been artificially inoculated with teliospores gathered in the field.

Although, in the above-mentioned examples, the teliospores may have represented a mixture of races, there is abundant evidence that, when pure cultures of physiologic races of normal spore color are selfed, they may produce color variants. Grayish-brown variants have occurred in the selfing of pure cultures of races of wheat stem rust collected in the field, and orange variants have occurred in the selfing of a pure culture of Race 6 of oat stem rust.

It is, however, from a study of crosses between color variants of physiologic races, rather than from selfing studies with field cultures, that some light has been thrown on the inheritance of this type of abnormality. Since the discovery of the first color aberrations (9), a number of crosses have been made between races of *P. graminis Tritici* that differed from each other in uredial color. A cross between a strain with orange uredia and one possessing grayish-brown uredia produced an F_1 hybrid race with normal (red) uredia (13). A selfing of the hybrid race produced an F_2 generation composed of cultures with red, grayish-brown, orange, and white uredia. Other crosses between orange and grayish-brown races have produced similar results. Selfings of F_2 and F_3 cultures have shown that the red cultures fall, genotypically, into four classes. Some are homozygous for red spore color; some produce red and orange progeny; others produce red and grayish-brown progeny; while still others produce all four color types; namely, red, grayish-brown, orange and



TEXT-FIG. 1. *Part of a progeny study of a cross between a race with grayish-brown uredia and a race with orange uredia, showing color segregation resulting from the selfing of red, grayish-brown, orange and white races. Of the F_2 cultures, only one grayish-brown, one orange, and one white culture were selfed.*

white. The grayish-brown strains when selfed fall into two classes. Some are homozygous for grayish-brown color; others produce grayish-brown and white strains. Similarly, the orange strains are either homozygous for orange color or produce orange and white cultures. The white strains, when selfed, produce only cultures with white uredia. Text-fig. 1 shows a typical progeny study of a cross between grayish-brown and orange races.

From a consideration of the behavior of the color variants in crosses and selfing studies it is clear that red spore color is dominant over grayish-brown, orange, and white, and that grayish-brown or orange spore color is dominant over white.

As color variants originate not infrequently from the selfing of physiologic races of normal color collected in nature, it is obvious that the genetic factors responsible for this difference in color are inherent in certain strains of the rust. Being recessive, these factors would have no visible effect while they are present in a heterozygous condition. When these factors are brought into a homozygous condition by selfing, their effect becomes apparent in uredial color deviating from the normal. A detailed scheme of the inheritance of urediospore color has been suggested by Johnson, Newton, and Brown (7).

The extreme rarity of strains of abnormal color in nature would suggest that such strains have a lower survival value than strains of normal color. At present, the reasons for this phenomenon can only be surmised. The spore-pigmentation characteristic of stem rust is possibly an ideal protective agent against the ultra-violet light produced by the sun as is, indeed, suggested by the work of Dillon Weston (2), who demonstrated that the urediospores of orange and white strains were more readily destroyed by artificially produced ultra-violet light than those of red or grayish-brown strains. Furthermore, it is possible that the urediospores of color variants are less viable than those of normal strains as has, in fact, been demonstrated by Newton and Johnson (9) for the first two color variants described by them. The germinability of the urediospores of these variants was considerably lower than that of rust spores of normal color. There is also reason to suppose that the urediospores of color variants are viable for a shorter period than spores of normal strains. This conclusion has been derived from experience with the storage of urediospores at about 10° C. and 50% relative humidity, under which conditions it is frequently found that, after a certain period of storage, the spores of the color variants fail to produce infection on wheat seedlings, whereas spores of normal color are still capable of causing infection.

The Occurrence of Strains Showing a Decrease in the Vigor of Sporulation

Another abnormal characteristic that has occasionally been noted in inbred races, but not in races collected in nature, is the tendency to form uredia which fail, partially or entirely, to rupture the epidermis of the wheat plant. Strains of this type have, for the sake of convenience, been designated as "subepidermal" (Plate I, Fig. 1). Although never collected in nature they have

originated from the selfing of two physiologic races collected in the field, namely, Race 21 and Race 36. In a selfing of the former race, one culture of a progeny of 35 cultures showed this characteristic; in a selfing of the latter race two cultures of a progeny of 23 cultures were subepidermal. It is evident, therefore, that the hereditary factors governing this condition are occasionally present in physiologic races collected in nature.

Subepidermal strains occur, however, more frequently in the selfing of cultures derived from crosses between physiologic races. In the F_2 generation of a cross between Races 36 (grayish-brown) and 9 (red), 7 out of 199 cultures were more or less subepidermal. Two of the F_2 cultures that formed pustules in a normal manner produced, when selfed, an F_3 progeny containing subepidermal strains. Of one of these, Race 36 (grayish-brown), 45 F_3 cultures were studied and classified as follows, according to the ability of the pustules to rupture the epidermis.

Normal pustule development	2
Very slightly subepidermal	5
Slightly subepidermal	7
Partially subepidermal	21
Entirely subepidermal	10

Although most commonly observed in races of wheat stem rust, subepidermal strains have also been noted, on one occasion, in selfing studies with physiologic races of oat stem rust. In the selfing of Race 6 (orange), 3 subepidermal cultures occurred in a progeny composed of 36 cultures. Race 6 (orange) itself was derived from the selfing of Race 7 (orange) which, in turn, originated from aecia on a barberry infected by teliospores collected in the field. If the teliospores from the field are considered as the F_1 generation, the subepidermal condition first became apparent in the F_4 generation, that is, after three consecutive selfings.

It is clear from the instances already cited that the occurrence of subepidermal strains is associated with the inbreeding of physiologic races. The formation of such strains does not, however, appear to be a necessary consequence of inbreeding. Many strains of wheat stem rust that have been selfed for several successive generations show no decrease in their vigor of sporulation. The explanation is more likely to be found in the genotypical characteristics of certain strains, the selfing of which tends to segregate and bring together the factors responsible for the subepidermal condition. Neither is the subepidermal condition necessarily associated with abnormal urediospore color, for many subepidermal strains have urediospores of normal color.

As no subepidermal strains have yet been selfed, nothing can be said at present concerning their breeding behavior.

The Occurrence of Strains Showing a Weakening of the Pathogenicity of the Rust

It might be expected that further inbreeding would have no effect on the pathogenicity in physiologic races that have already proved homozygous

for pathogenicity, as judged by the infection types produced on the differential wheat varieties. That is to say, the progeny of further selfings should consist of cultures pathogenically identical with each other and with the parent culture. Although this expectation has been borne out in the majority of selfing studies with homozygous races there have, nevertheless, been exceptions in which unexpected results were obtained. The exceptions thus far noted have represented a change in one direction, namely, towards a lower level of pathogenic vigor, such as a change from a "4" type of infection to a "3" $\pm$ or an "x" type.

One of the selfing studies carried out with Race 9 furnishes an example of such a degeneration of pathogenic vigor. Race 9, when originally selfed, proved homozygous for pathogenicity. The 40 F_2 cultures studied were alike and produced a "4" type of infection on the varieties Mindum, Spelmar, and Vernal (12). Other selfings on a smaller scale confirmed the conclusion that the original culture was homozygous for pathogenicity. One of the F_2 cultures derived from the original selfing of this race was retained and kept in culture in the greenhouse for several years, with intervals of storage in a refrigerator at 10° C. and a relative humidity of 50%. Four years after the original selfing, adult wheat plants were inoculated with this culture with the object of producing teliospores for a selfing study. The usual checking of the purity of the race prior to teliospore formation showed no trace of contamination. When, however, this F_2 culture was selfed it produced, instead of a progeny composed entirely of Race 9, an F_3 generation composed of 15 cultures of Race 9, 5 cultures of Race 17, 19 cultures of Race 29, 9 cultures of Race 85 and 11 cultures of Race 149, a hitherto undescribed race. Other peculiarities were noted such as the fact that one of the 15 cultures of Race 9 produced uredia which were almost entirely subepidermal while two others produced uredia described as Mars Yellow in color.

A comparison of the infection types produced by the five physiologic races that arose from this selfing will show that the deviation from Race 9 is in every instance a degradation of infection type from a "4" type to an "x" type, and even to a "1" type in the case of the variety Vernal (Table I). It should be noted also, that the "x" type of infection produced by different cultures, identified as the same physiologic race, varied considerably in vigor. Thus two cultures, both identified as Race 149, showed a marked difference in the vigor of the "x" type of infection produced on Mindum and Spelmar (Plate I, Fig. 2). The classification of the cultures into five physiologic races does not, therefore, give an adequate idea of the variation in pathogenicity occurring in the progeny of this selfing. Nevertheless the variation in pathogenicity is not of the kind usually obtained in the selfing of a heterozygous race. Although the progeny was divided into five physiologic races, the pathogenic differences of these are slight except on the variety Vernal that clearly differentiates Races 17 and 29 from the other three races. With this exception, the races derived from this selfing bear a close resemblance to the parent Race 9. If the pathogenic differences in the progeny were due to an

undetected contamination by some other race, a more distinct variation in pathogenicity would have been expected.

It is difficult to account for the above-mentioned facts without having recourse to the supposition that a mutation or more probably a series of mutations had occurred in this race in the interval between the original and the present selfing. As this race gave rise to the first known mutant in stem rust, a color mutant also described as Race 9 (9), it is possible that mutations recur in this race from time to time. If this view is accepted it is clear that the degradation of pathogenicity and the development of other abnormal characteristics are results of inbreeding only in so far as inbreeding tends to segregate the hereditary factors changed by mutation. As these characteristics were not expressed in the parent race, the factors governing them are probably recessive.

Evidence of the decrease of pathogenic vigor in inbred strains has recently been secured in a different manner. Experiments on the effect of high temperatures on the rust development of physiologic races of wheat stem rust have shown that inbred strains are more sensitive to high temperatures than physiologic races collected in nature (6). Most inbred races failed to produce normal types of infection on susceptible wheat varieties when the mean daily greenhouse temperature exceeded 80° F. At higher temperatures the ordinary "4" type of infection of these races was replaced by a "3" type or an "x" type of infection or even by necrotic flecks (Plate I, Fig. 3). Races collected in the field were less affected by temperature. Of five such races that were tested, four showed little or no displacement of their ordinary infection type, when subjected to a mean daily temperature of 95°–99° F., whereas one race tended to produce an "x" type of infection at mean daily temperatures of 85°–89° F. and failed entirely to develop at higher temperatures. It is evident that differences in response to temperature exist among races collected in nature but, even after allowance is made for such differences it is clear that inbred races are less tolerant of high temperatures than those collected in the field.

If inbreeding occurs to any extent in nature, it is likely that strains similar to those produced in the laboratory would appear. If they do appear it is, however, probable that they have a low survival value and are consequently seldom collected.

TABLE I

INFECTION TYPES PRODUCED BY THE SELFED F_2 CULTURE OF RACE 9 AND BY THE FIVE PHYSIOLOGIC RACES OCCURRING IN THE F_3 PROGENY

Physiologic race	Little Club	Marquis	Kanred	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli
F_2 culture—9	4	3+	0	3+	4−	3+	4	4	4−	3+	3+	1
F_3 culture—9	4	3±	0	3−	3+	3+	3+	4−	3+ to x	3	3±	1−
F_3 culture—17	4	4−	0	3+	4−	4−	4−	4	3+	3+	1	1−
F_3 culture—29	4	4−	0	3±	4−	x	x	x+	3+	3+	1±	1
F_3 culture—85	4	4−	0	3	4−	4−	4−	4−	3±	3+	x	1−
F_3 culture—149	4	x+	0	3−	x+	x	x	x	x	x	x	1−

The Effect of Inbreeding on the Pycnial and Aecial Stages

The Loss of Ability to Produce Aecia on the Barberry

The effect of inbreeding has been manifested not only in peculiarities associated with the uredial and telial stages but also in abnormalities of the pycnial and aecial stages. The loss of ability of certain strains to produce aecia and the development of uredia and telia on the barberry by some of these strains are two such abnormalities. The last-mentioned abnormality has already been reported briefly (11). As the production of uredia and telia on the barberry appears to be associated with the suppression of aecia, it is necessary to deal first with the latter phenomenon.

Strains of stem rust that have partly or entirely failed to produce aecia have been known by the writers since 1931. In that year several barberry plants were inoculated by the sporidia of teliospores of Race 1 (white), an F_2 culture derived from a cross between Races 9 (orange) and 36 (grayish-brown). The pycnial pustules resulting from these inoculations produced only traces of pycniospore-containing nectar. Although nectar was intermixed on several hundred pustules, only one of these produced normal aecia while three other pustules developed rudimentary aecia containing only a few aeciospores. Difficulties were also experienced in obtaining aecial formation by the application of nectar from other races to the pycnia of the white race. When, however, composite nectar of Race 95 was transferred to 45 pustules of Race 1 (white), aecia developed in five of these. The application of foreign nectar was therefore somewhat more efficacious in bringing about aecial formation than the intermixing of the nectar of the white race itself, but, nevertheless, the proportion of pustules producing aecia was abnormally low. Another F_2 culture derived from the same cross, Race 11 (red), behaved in a similar manner. No aecia resulted from the intermixing of the nectar of more than 50 pustules. In 1934 and 1935 a similar behavior was noted in four white races in the F_3 and F_4 generations of another cross—Race 52 (grayish-brown) $\times$ Race 9 (orange). The pycnia formed by these races produced only minute quantities of nectar which showed a tendency to dry up soon after its production. Pycniospores were present but less abundantly than in normal nectar. Such pycnial nectar as was produced by each of these races was intermixed and a record was kept of the number of pustules that produced aecia. Race 15 (white), an F_3 culture, produced aecia in only 2 out of a total of 140 pustules. Race 52 (white), another F_3 culture, produced no aecia in a total of 72 pustules. Race 9 (white), an F_4 culture, produced aecia in 13 of a total of 88 pustules. Race 57 (white), another F_4 culture, formed aecia in 17 of a total of 72 pustules.

The failure of these races to produce aecia can not, at present, be satisfactorily explained. A complete failure to form aecia, as in Race 52 (white), can not be solely attributed to the sparseness of pycniospore-containing nectar for, as many compound pustules were present, there should have been considerable aecial formation resulting from the coalescing of haploid pustules of opposite sexes even if the pycniospores were not functional. Neither can

the suppression of aecia be attributed to the loss of pigment in the urediospores, as might be concluded from the fact that most of the above-mentioned races formed white uredia. The two phenomena are not necessarily associated, as some white strains produce aecia abundantly.

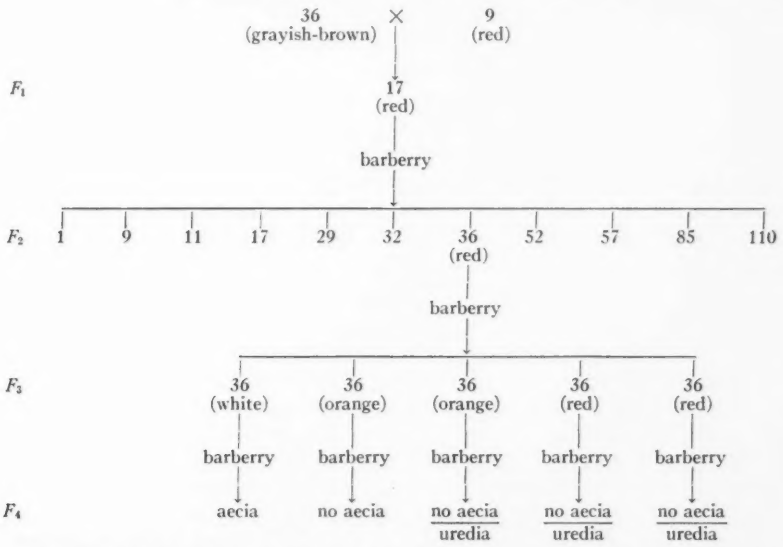
The Development of Uredia on the Barberry by Some Strains Which Have, Partially or Entirely, Lost the Ability to Produce Aecia

During the winter 1936–37, barberry plants were inoculated with five F_3 cultures derived from a cross between Races 36 (grayish-brown) and 9 (red). These five cultures were descended from the same parent, an F_2 culture which had been identified as Race 36 (red). This F_2 culture appeared homozygous for pathogenicity, as all its F_3 progeny (composed of 35 cultures) were identified as Race 36. The five F_3 cultures selected for further study differed, however, in uredial color; one being white, two orange, and two red. They differed also in their behavior on the barberry. The white culture produced pycnial nectar in abundance and formed aecia in a normal manner. The other four cultures produced very small quantities of pycnial nectar which, however, contained pycniospores, but they formed no aecia either as a result of intermixing this nectar or by the application to their pustules of the nectar of other races. One of the orange cultures, and both of the red cultures produced uredia and telia on the barberry. The interval between inoculation and the production of uredia varied somewhat in different infection trials. The shortest interval noted in any trial was 35 days, the longest 57 days, the average interval being from 44 to 47 days. Text-fig. 2 shows the origin of the cultures and the spore forms produced by them on the barberry.

In most of the infection tests, only a relatively small proportion of the pustules produced uredia, and this proportion varied considerably in different tests. In one test 50 pustules out of a total of 129 were found to contain uredia. Of these 50 pustules at least 21 bore telia as well as uredia. In a later infection trial, on a different barberry plant, uredia were found on only 10 pustules out of a total of about 150.

In certain infection tests attempts were made to determine what proportion of the uredia-bearing pustules were single and compound pustules respectively. This, however, could not be done with any degree of certainty. In the infection test mentioned above, in which 50 uredia-bearing pustules were found, it was judged that 10 of these were single pustules, 31 compound, while 9 could not be determined definitely as either single or compound. The majority of the uredia were, therefore, borne on compound pustules formed by the coalescing of two or more adjacent infections.

This raises the question of whether the uredia-bearing pustules arose from the fusion of mycelia of opposite sexes. The fact that 31 out of 60 pustules that were obviously compound produced uredia whereas 29 failed to do so would suggest that mycelia of opposite sexes interacted. On the other hand 10 pustules that were judged to be single produced uredia. It is not, however,



TEXT-FIG. 2. *Pedigree of the strains of Race 36 that produce urediospores and teliospores on the barberry.*

altogether safe to assume that these were of monosporidial origin and that the urediospores arose on homothallic mycelia.

The uredia were most abundant on the upper surface of the leaves but were not infrequently found on the lower side also. Plate I, Fig. 4 shows uredia of Race 36 (red) on the upper surface of a barberry leaf. Urediospores and teliospores were both normal in appearance. The urediospores germinated in their characteristic manner and infected wheat seedlings on which normal uredia were produced, but were unable to infect the barberry. All cultures which have, thus far, been established by these urediospores have been identified as Race 36. They are therefore pathogenically identical with the parent culture.

It is evident that in these four strains of Race 36 a reduction has taken place from a full cycle (0, I, II, III) to what might be considered a brachy-cycle (0, II, III). For the sake of convenience these strains will, therefore, be referred to as "brachy-strains". When viewed in this light the uredia formed by them on the barberry might be considered as primary uredia. Until these strains have been submitted to another selfing it is, however, impossible to say whether this tendency to a brachy-cycle is a permanent or a transient characteristic.

Uredia and telia have also been produced on the barberry by a culture of Race 21 collected in the field at Indian Head, Sask., in 1934. Teliospores of this culture were produced in the greenhouse in the spring of 1936 and barberries were infected by these in January, 1937. When pustules developed

on the barberry, it was observed that only about one-half of them produced pycnial nectar in a normal manner. The remaining pustules were almost white in color. Many produced no pycnia or only rudimentary ones. Some, however, contained scattered pycnia which eventually produced small quantities of pycniospore-bearing nectar. Many of the white pustules began to produce urediospores and teliospores about six weeks after inoculation (Plate I, Fig. 5). The urediospores, like the ones produced on the barberry by the cultures of Race 36, were incapable of infecting the barberry but infected wheat seedlings readily.

Three cultures were established from uredia produced on three different barberry leaves with the object of determining the physiologic races contained in them. Two of these were pure cultures of Race 1 and one was a mixture of Races 1 and 17. Races 1 and 17 also predominated in cultures originating from the aecia produced by Race 21. In a study of 36 cultures, each originating from a single aecium of Race 21, selected at random, Race 1 occurred nine times, Race 17 twenty times, Race 21 once, Race 78† five times, and race 136† twice. Although the cultures originated by the uredia were too few to permit a satisfactory comparison with those initiated by the aecia, the similarity of the physiologic races derived from the two sources would suggest that the aeciospores and the urediospores are genotypically alike.

Attempts were made to discover whether or not the pycniospores of the races that produced uredia on the barberry had lost their function. The loss of function of the pycniospores would not, it is true, explain the development of uredia on the barberry, but it might conceivably be the cause of the suppression of the aecia. If the application of nectar of these races to haploid pustules of other races resulted in the development of aecia, proof would have been obtained that the pycniospores were still functional. Accordingly composite nectar of Race 36 (red)—a race that formed uredia but no aecia on the barberry—was transferred to six haploid pustules of Race 36 (white) which has regularly produced aecia in a normal manner. When selfed, this race, like all white races, develops aecia and aeciospores of a pale buff color. When pycniospores of a red race—that is, a race producing red uredia—are used to diploidize the pustules of a white race, the resulting aecia are orange in color and contain orange aeciospores which give rise to red uredia when they infect wheat seedlings. The production of orange aeciospores in any of the six pustules to which nectar of Race 36 was applied would therefore be a proof of the functioning of the pycniospores of this race. Aeciospores of this color were, in fact, developed in four out of the six pustules. Inoculation of wheat seedlings by these aeciospores gave rise to red uredia, a fact which proved conclusively that the pycniospores of the red race, though incapable of initiating aecia in a selfing of that race, were capable of doing so when applied to the haploid pustules of the white race. Although the pycniospores were thus able to diploidize pustules of the white race, the aecia produced were

†*It should be remarked that Races 78 and 136 differ but slightly in pathogenicity from Races 17 and 1.*

scarcely more than rudimentary structures that barely succeeded in breaking through the epidermis of the barberry leaf. As the white race, when selfed, produces vigorous aecial development the lack of vigor noted in this case is clearly attributable to the red race.

In a similar manner it was demonstrated that the pycniospores produced by the white pustules of Race 21 were able to diploidize pustules of Race 1 (white). The application of a composite nectar from two pustules of Race 21 to four haploid pustules of Race 1 (white) produced orange aecia and aeciospores in all four pustules. These aeciospores, in turn, gave rise to red uredia on wheat seedlings. The four uredial cultures thus initiated were all identified as Race 17. The aecia in this instance were normal in size and structure.

The ability of the pycniospores of these strains to induce aecial formation in the white race suggests strongly that the suppression of aecia is not due to non-function of the pycniospores. This conclusion is supported by the fact that neither the pycniospores of Race 1 (white) nor those of other races appear capable of bringing about the formation of aecia when they are applied to the pycnia of the brachy-strains.

Discussion

Sufficient evidence has been presented in the present paper to demonstrate that the inbreeding of physiologic races gives rise to various abnormal characteristics that are rarely, if ever, encountered in nature. Abnormal characteristics are not, however, an inevitable consequence of inbreeding, for many inbred strains show no deviation from the normal. These abnormalities must arise in one of two ways. Either they originate through mutations that occur during the passage of the rust through the barberry, that is, during the selfing process, or they arise from a recombination of genetic factors already present in the physiologic races before they were selfed. In either case the abnormalities would be attributable to mutation. If the aberrations arise from the recombination of already existing genetic factors it would follow that these genetic factors, though present in the rust, do not produce any observable effects on the rust so long as it remains in the uredial stage. Such, indeed, would be the case if these genetic factors were recessive. They might then have originated through mutations which had taken place at some time or other in the past history of the rust; and their effects would not become visible until they had been segregated and recombined in the process of selfing.

While it is not easy to determine which of the two above-mentioned alternatives plays the more significant part in the origin of abnormal rust characteristics, it can scarcely be doubted that the process of mutation is actively at work in the rust fungi. This has been amply demonstrated in recent years by investigators of certain of the cereal rusts. Mutations for uredial color have been reported in stem rust of wheat by Newton and Johnson (9) and Waterhouse (16). Mutations for pathogenicity have been recorded by Stakman, Levine, and Cotter (15) in wheat stem rust, by Gassner and Straib (3) in *Puccinia glumarum* (Schmidt) Erikss. & Henn., and by Roberts (14) in

Puccinia triticina Erikss. An aberrant physiologic race of *P. triticina*, differing from other known races in length of incubation period, spore color, and size of uredia, has been described by Johnston (8) who suggested the possibility of its origin by mutation.

In view of the number of authentic mutations already recorded in cereal rusts, it seems probable that mutation in the rust fungi is a constantly recurring phenomenon which plays a part in the origin of new genetic factors that do not always find expression while the rust remains in its dikaryotic (uredial) stage. As, however, stem rust is a heterothallic rust, a continuous interchange of genetic factors is taking place between the various races of which it is composed, an interchange accomplished by the intermixing of the pycnial nectar of different haploid pustules and the fusion of pustules arising from mycelia of opposite sexes (1). Consequently most physiologic races are in a heterozygous state. This heterozygosity has probably been increased by numerous mutations of a recessive type, which have taken place in the past, and which have been distributed among physiologic races through the processes just indicated. It is probable that many of the abnormalities that have appeared through the selfing of physiologic races are the result of such recessive mutations which are masked by dominant factors, and thus do not produce visible effects until they are brought together in a homozygous condition. It has been demonstrated in crossing and selfing studies that some of the abnormalities reported in the present paper behave as if they were governed by recessive factors. Thus grayish-brown and orange urediospore color is dominant over white, whereas red spore color is dominant over grayish-brown, orange, and white. While the inheritance of other abnormalities, such as the inability of the uredia of certain strains to rupture the wheat epidermis, has not been studied in detail it would seem probable, owing to their not infrequent occurrence in the selfing of perfectly normal rust strains, that they are also governed by recessive genetic factors. If this assumption is correct and generally applicable to abnormalities in stem rust, it would follow that the abnormal strains are homozygous for the genetic factors responsible for their deviation from the normal. In the laboratory this homozygosity is accomplished by inbreeding for several successive generations, a process which probably occurs rarely in nature. When abnormal types of rust do occur in nature they probably have a low survival value and would not be observed frequently.

The production of urediospores and teliospores on the barberry is, perhaps, the most unexpected of the abnormalities described in this paper. Any attempt at explaining this phenomenon must take into account the suppression of aecial formation which, thus far, has always accompanied it. One possible reason for the failure of a race to produce aecia would be the non-function of the pycniospores. It has been pointed out that the brachy-strains of Race 36 cannot be induced to form aecia through either an inter-mixing of their own nectar or the application of the nectar of other races. Nevertheless, the nectar from these strains, when applied to haploid pustules of other races, is capable of bringing about diploidization which results in some aecial formation.

The same holds true for the pycniospores of the very sparse nectar of the white pustules of Race 21. Obviously, therefore, the pycniospores are still functional.

Another possible reason for the failure of these strains to form aecia might be the loss of a sex factor. However, if the factor for one sex were lost the application of a composite nectar of other races—nectar containing pycniospores of both sexes—should bring about aecial formation in approximately one-half of the haploid pustules, which, as already pointed out, does not occur. The factor for at least one sex must be present, otherwise the pycniospores of the brachy-strains would not be capable of diploidizing the haploid mycelia of other races.

It would seem then that the only explanation that can be advanced is to assume that a genetic factor (or factors) governs the production of aecia and to ascribe the failure of aecial formation in these strains to a loss of this factor or factors. That this factor acts as a dominant is suggested by the fact that the pycniospores of the brachy-strains do not suppress aecial formation when they are used to diploidize haploid pustules of another race. The lack of ability to produce aecia is, therefore, a recessive condition, or at least not a dominant one. With the loss of the factor governing aecial production, sporulation takes the alternative form and uredia are produced.

Regarded from the point of view of phylogeny the occurrence of uredia and telia of *Puccinia graminis* on the barberry may have some significance in explaining the origin of short-cycled rusts from heteroecious long-cycled forms. According to Jackson (5) microcyclic forms arise in most cases from the haploid generation of heteroecious eu-forms through the replacement of the aecia by telia. Short-cycled species which appear to have originated in this manner are known as "correlated" species. Thus, for example, *Puccinia Grossulariae* (Pers.) Lag., with aecia on *Ribes* and uredia and telia on *Carex*, may have given rise to *Puccinia Parkerae* Diet. and Holw., a micro-form with telia on *Ribes*. Similarly, as pointed out in private correspondence by Dr. G. R. Bisby, *Puccinia graminis* might, in theory, have a microcyclic, correlated species with telia on *Berberis vulgaris*. The development of uredia and telia on the barberry by Races 36 and 21 may perhaps be considered as a tendency on the part of *Puccinia graminis* towards the formation of such a correlated species. This interpretation would lend support to the theory that correlated microcyclic forms were derived, through reduction, from heteroecious long-cycle forms.

It appears doubtful whether the occurrence of uredia and telia on the barberry throws any light on the relative primitiveness of heteroecism and autoecism. A heteroecious rust has apparently made an attempt—but an unsuccessful one—to become autoecious; unsuccessful because the urediospores produced on the barberry still retain their normal physiological nature and infect grasses, but not the barberry. It might be argued that this phenomenon supports the idea that heteroecism is a more primitive condition than autoecism, that this attempt at autoecism represents an inherent tendency in

heteroecious rusts to become autoecious. But possibly it might also be argued that a heteroecious rust was here attempting to revert to a more primitive autoecious condition.

References

1. CRAIGIE, J. H. An experimental investigation of sex in rust fungi. Phytopathology, 21 : 1001-1040. 1931.
2. DILLON WESTON, W. A. R. The effect of ultra-violet radiation on the urediniospores of some physiologic f ms of *P. graminis tritici*. Sci. Agr. 12 : 81-87. 1931.
3. GASSNER, G. and STRAIB, W. Ueber Mutationen in einer biologischen Rasse von *Puccinia glumarum tritici* (Schmidt) Erikss. und Henn. Z. indukt. Abst. Vererbungsl. 63 : 154-180. 1932.
4. GORDON, W. L. A study of the relation of environment to the development of the uredinial and telial stages of the physiologic forms of *Puccinia graminis avenae* Erikss. and Henn. Sci. Agr. 14 : 184-237. 1933.
5. JACKSON, HERBERT S. Present evolutionary tendencies and the origin of life cycles in the Uredinales. Memoirs Torr. Bot. Club, 18 : 1-108. 1931.
6. JOHNSON, THORVALDUR and NEWTON, MARGARET. The effect of high temperature on uredial development in cereal rusts. Can. J. Research, 15 : 425-432. 1937.
7. JOHNSON, T., NEWTON, MARGARET and BROWN, A. M. Further studies of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici*. Sci. Agr. 14 : 360-373. 1934.
8. JOHNSTON, C. O. An aberrant physiologic form of *Puccinia triticina* Erikss. Phytopathology, 20 : 609-620. 1930.
9. NEWTON, MARGARET and JOHNSON, THORVALDUR. Color mutations in *Puccinia graminis tritici* (Pers.) Erikss. and Henn. Phytopathology, 17 : 711-725. 1927.
10. NEWTON, MARGARET and JOHNSON, THORVALDUR. Specialization and hybridization of wheat stem rust, *Puccinia graminis tritici*, in Canada. Dominion of Canada, Dept. of Agr. Bull. 160, N.S. 1932.
11. NEWTON, MARGARET and JOHNSON, THORVALDUR. Production of uredia and telia of *Puccinia graminis* on *Berberis vulgaris*. Nature, 139 : 800-801. 1937.
12. NEWTON, MARGARET, JOHNSON, T. and BROWN, A. M. A preliminary study of the hybridization of physiologic forms of *Puccinia graminis tritici*. Sci. Agr. 10 : 721-731. 1930.
13. NEWTON, Margaret, JOHNSON, T. and BROWN, A. M. A study of the inheritance of spore colour and pathogenicity in crosses between physiologic forms of *Puccinia graminis tritici*. Sci. Agr. 10 : 775-798. 1930.
14. ROBERTS, FLORENCE M. The determination of physiologic forms of *Puccinia triticina* Erikss. in England and Wales. Ann. Appl. Biol. 23 : 271-301. 1936.
15. STAKMAN, E. C., LEVINE, M. N. and COTTER, R. U. Origin of physiologic forms of *Puccinia graminis* through hybridization and mutation. Sci. Agr. 10 : 707-720. 1930.
16. WATERHOUSE, W. L. Australian rust studies I. Proc. Linn. Soc. N.S.W. 54 : 615-680. 1929.

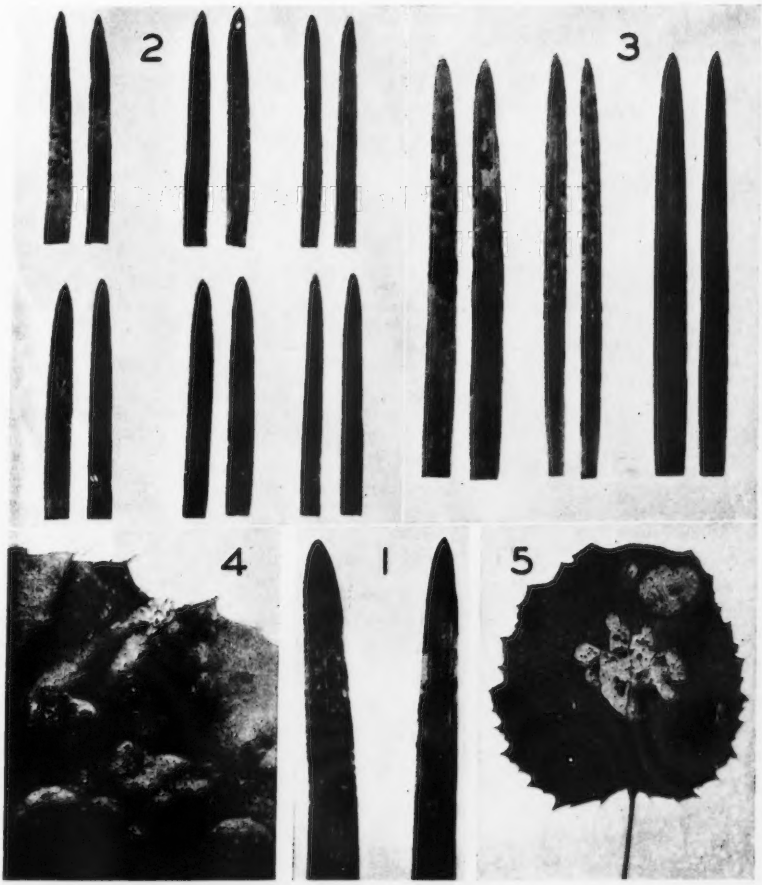


FIG. 1. *Infection types produced on wheat seedlings by two physiologic races of P. graminis Tritici. Left: "Subepidermal" pustules produced by Race 17 (cinnamon brown). Right: Normal pustules produced by Race 56 (red).* FIG. 2. *Infection types produced on seedlings of three wheat varieties by two cultures of Race 149. Top row: left to right, Marquis, Spelmar, and Einkorn infected by Culture No. 9. Bottom row: left to right, the same varieties infected by Culture No. 41 which produces a less vigorous pustule development.* FIG. 3. *The effect of high temperatures (mean maximum 99.6° F., mean minimum 66.8° F.) on the infection types produced by two physiologic races of P. graminis Tritici on Little Club seedlings. Left: Race 48—unaffected by the high temperature. Right: Race 36 (Sudan Brown)—strongly affected by the high temperature. Centre: Race 36 (Sudan Brown)—at approximately normal temperatures.* FIG. 4. *Upper surface of a barberry leaf showing uredia on pustules of P. graminis Tritici, Race 36 (red). Enlarged.* FIG. 5. *Lower surface of a barberry leaf showing a compound pustule of P. graminis Tritici Race 21. One component of the pustule contains aecia, the other small uredia.*

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VARIATIONS IN GROWTH INDICES OF *VENUS MERCENARIA* L. FROM WIDELY SEPARATED ENVIRONMENTS OF THE ATLANTIC COAST[1]

BY CURTIS L. NEWCOMBE[2], SARAH J. THOMPSON[3], AND HERMAN KESSLER[4]

Abstract

Linear and shell-weight indices of size in *Venus mercenaria* from three widely separated regions of the Atlantic Coast, namely, Gulf of St. Lawrence, Chesapeake Bay and North Carolina have been studied. The maximum variations revealed in the "*b*" values for the linear dimensions of *Venus* from the different regions are appreciable, whereas differences in the actual widths and thicknesses of corresponding lengths are not considered significant. The shell weights of specimens collected in the northern latitude of the Gulf of St. Lawrence are heavier than those from the warmer waters of the Chesapeake Bay and North Carolina. The length–shell-weight relations for the three regions are: Gulf of St. Lawrence, Wt. = 0.00000214 $L^{3.003}$; Chesapeake Bay, Wt. = 0.00000171 $L^{3.032}$; and North Carolina, Wt. = 0.00000108 $L^{3.151}$. No significant correspondence exists between linear growth dimensional ratios and known environmental influences, whereas shell weights seem to correspond with the temperature factor, an inverse relation being the result.

Introduction

Comparatively few studies have been made on the variations in growth indices of pelecypod molluscs from widely separated regions. Work in this field has dealt chiefly with the growth dimensional ratios of a certain species collected from a single locality (2, 7). The relative value of the several indices for expressing size and their relations to one another have been definitely determined for several animals (*Cf.* 4, 6). The extent of the variations that occur in a single species throughout its geographical range has received very little attention.

In a comparative study of the linear and weight indices of *Mya arenaria* L., results have been obtained that indicate a close correspondence between temperature and shell weight, heavier shells being formed in the colder regions (5). With the idea of enlarging the available data dealing with this subject, upon which opinion is by no means unanimous, the present study was undertaken.

Variations in the linear indices of length, width, and thickness as well as differences in the shell weights of *Venus mercenaria* in the Gulf of St. Lawrence, Chesapeake Bay, and North Carolina regions have been investigated.

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A co-operative study from the Chesapeake Biological Laboratory and the Department of Zoology of the University of Maryland.

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Dry body weights of the soft parts were taken but are not included in this report owing to the uncertain nature of the effect of the preservative on the actual body weight. Brief mention is also made of the environmental factors that seem to distinguish the three regions.

The specimens were kindly provided by Dr. A. W. H. Needler, Assistant-in-Charge of the Prince Edward Island Biological Station; Dr. H. F. Prytherch, Director, U.S. Bureau of Fisheries Laboratory, Beaufort, N.C.; and Mr. G. T. Elliott, of Hampton, Va., for whose assistance we are very grateful. Acknowledgment is made to Dr. R. V. Truitt, Director of the Chesapeake Biological Laboratory, for his co-operation in all phases of the study.

Methods

The linear dimensions, length, width, and thickness, were measured with a vernier caliper reading to 0.1 mm. The length measurement (L) is the greatest anterior-posterior dimension; the width (W) represents the greatest radius with the umbo as a centre; and the thickness measurement refers to the greatest distance between the two valves of the tightly closed animal placed in a lateral position.

All the collections were shipped to the laboratory in a living condition, dried to constant weight, and the individual shells weighed after the body parts had been removed. In all, 212 specimens were examined, the numbers from the individual regions being as follows: Gulf of St. Lawrence, 38; Chesapeake Bay, 38; and North Carolina, 136. Because of unfavorable field conditions at the time the collections were taken, it was not possible to obtain greater numbers from the Gulf of St. Lawrence and Chesapeake Bay regions.

Constants of the equations*

$$(1)\quad W = a + bL$$
$$(2)\quad T = d + eL$$

expressing the relations of both width (W) and thickness (T) to length (L) were obtained by the method of Lipka (3, p. 259). It was found that, within the length range studied, the relation between the logarithm of the length and that of shell weight is expressed by a straight line, hence the application of Huxley's power law, the form of the function being $Wt = cL^k$. The values of the constants c and k were calculated by Lipka's method. c is a constant denoting the value of Wt when $L = 1$, and has been referred to as the fractional coefficient. The constant k of the so-called simple heterogony formula represents the ratio of the relative growth rate of the shell weight to the relative growth rate of the length. The expression "relative growth rate" implies the rate of growth per unit dimension.

Results

The growth dimensional ratios obtained for the North Carolina collection ($N = 136$) are presented in Table I and may be readily compared with

** a or d is a y intercept or a mathematical value of the width (W) when the length (L) is zero. This definition has no biological counterpart since it is necessary to have a length dimension before the width may be estimated.*

b or e is the slope of the line or the absolute increment of width corresponding to each unit increment of length.

corresponding ratios for the two remaining regions. It is seen that the "b" values (length-width) for the Chesapeake Bay ($N = 38$) and Gulf of St. Lawrence ($N = 38$) represent the maximum difference. A comparison of the length-thickness ratios of the same collections has shown that no significant difference in the "b" values obtains (Table I).

TABLE I

CONSTANTS OF THE FUNCTIONS $W = a + bL$, $T = d + eL$ AND $Wt = cL^k$ IN WHICH W = WIDTH, T = THICKNESS, Wt = WEIGHT, AND L = LENGTH

Dimensional ratios	Constants	Gulf of St. Lawrence	Chesapeake Bay	North Carolina
Length to width	b	0.931	0.772	0.852
	a	−2.876	2.274	1.395
Length to thickness	e	0.560	0.583	0.570
	d	0.378	−0.634	−0.631
Length to shell wt.	k	3.003	3.032	3.151
	c	0.00000214	0.00000171	0.00000108

Comparison of the actual widths and thicknesses corresponding to the same lengths for the three widely separated regions shows that the greatest difference exists between the Chesapeake Bay and the Gulf of St. Lawrence clams, namely 12% for specimens 80 mm. in length. Values for the North Carolina and Gulf of St. Lawrence specimens are very similar (Figs. 1, 2).

The k values, which represent the ratios of the relative growth rates of shell weights as compared with lengths for the different regions, show that a maximum variation (5%) exists between the Gulf of St. Lawrence and North Carolina regions (Table I). The ratio found for the former group is very close to that obtained for the Chesapeake specimens. A noticeable variation exists in the actual shell weight corresponding to a given length. The differences are more conspicuous in the smaller specimens. The maximum variation exists between the specimens from the Gulf of St. Lawrence and those from North Carolina, clams 20 mm. long from the former region being 32% heavier and those 50 mm. long being 12% heavier. The Chesapeake Bay specimens bear a close similarity to those of North Carolina, the smaller ones being relatively heavier and the larger ones somewhat lighter.

Discussion

The data upon which this discussion is based were gathered from a study of specimens collected in the Gulf of St. Lawrence, Chesapeake Bay and North Carolina regions. The observations made are treated from the standpoint of regional variations in indices of size and in environmental conditions. There is little available information concerning the environmental factors operating in these regions. Temperature appears to be the factor that may

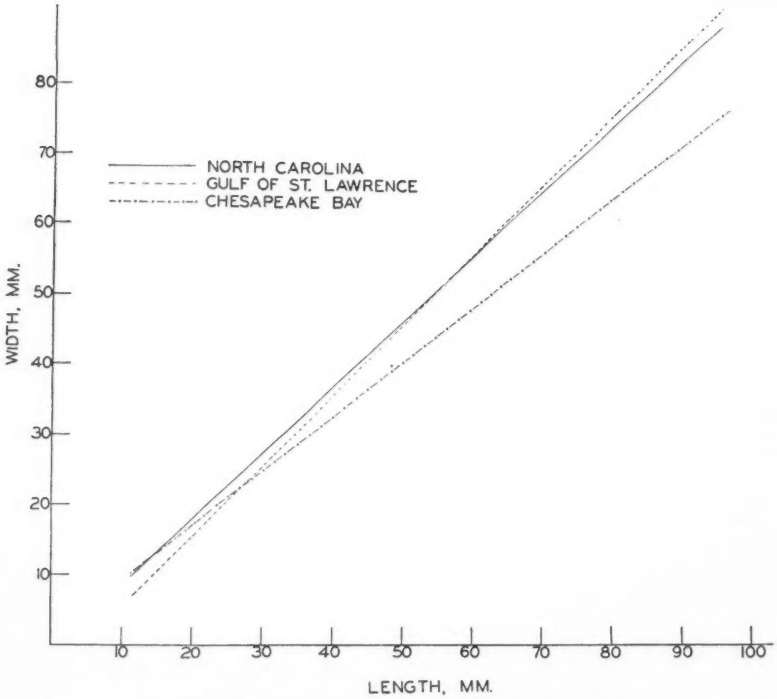

FIG. 1. *Showing length-width relations of Venus mercenaria from three regions of the Atlantic Coast. Gulf of St. Lawrence, $W = -2.876 + 0.931\ L$; Chesapeake Bay, $W = 2.274 + 0.772\ L$; North Carolina, $W = 1.395 + 0.852\ L$.*

influence the growth indices of *Venus mercenaria* (*Cf.* 5). The variations in soil and salinity do not seem sufficiently great to warrant serious consideration. Mean daily water temperatures during July and August in the region of Malpeque Bay (Gulf of St. Lawrence) average about 20° C. in comparison with values of about 26° C. in the section of the Chesapeake where the collection was taken* (1). Corresponding values for the North Carolina area are probably three degrees higher.

The results of this study seem to indicate a fairly close agreement with respect to the linear indices of the specimens studied. As is noted above, the "*b*" value for the length-width ratio of the Chesapeake collection is lower than that for the two remaining regions and seems to constitute one exception. Newcombe and Kessler (5) found that no significant variation exists in the linear growth dimensional ratios of *Mya arenaria* collected from different latitudes on the Atlantic Coast. This conclusion appears to lend support to the contention that a similar uniformity exists in *Venus mercenaria*.

* *Accurate records taken regularly are not available; hence values used are estimations based on irregular readings by different people during several seasons.*

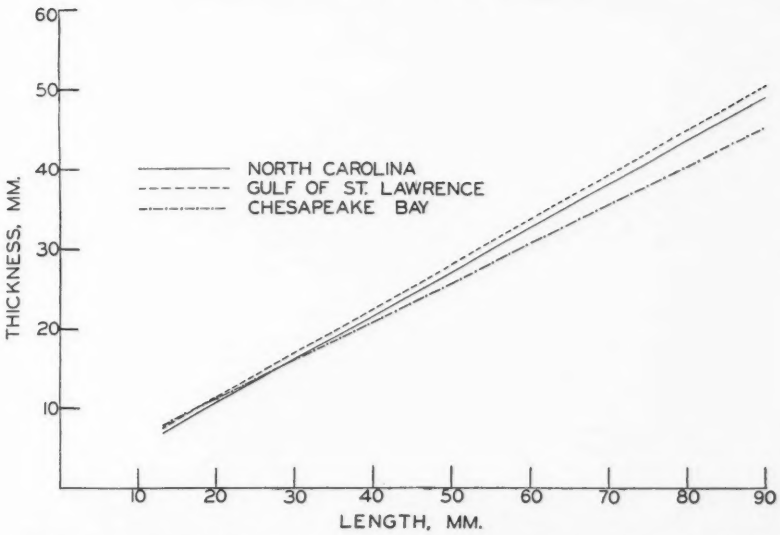

FIG. 2. *Showing length-thickness relation of Venus mercenaria from three regions of the Atlantic Coast. Gulf of St. Lawrence, $T = 0.378 + 0.560\,L$; Chesapeake Bay, $T = -0.634 + 0.583\,L$; North Carolina, $T = -0.631 + 0.570\,L$.*

It seems significant that the shell weights of *Venus* collected in the northern latitude of the Gulf of St. Lawrence are heavier than those of the two southern regions. There is added interest in view of the fact that specimens of *Mya arenaria* grown in the cold waters of the Bay of Fundy possess heavier shells than those living in the warmer waters of the Chesapeake Bay. On the other hand, specimens of *Venus mercenaria mortensii*, inhabiting the warmer waters of the Florida coast and considered by some systematists to be the same as the Chesapeake Bay form, possess distinctly heavier shells. In view of this fact, the inverse relation between shell weight and temperature obtained in this study seems quite significant.

References

1. COWLES, R. P. A biological survey of the offshore waters of Chesapeake Bay. U.S. Bur. Fisheries Document 1091. 1930.
2. GALTSOFF, PAUL S. The weight-length relationship of the Hawaiian Pearl Oyster, *Pinctada* sp. Am. Naturalist, 65 : 423-433. 1931.
3. LIPKA, JOSEPH. Graphical and mechanical computations. John Wiley and Sons, Inc., New York. 1918.
4. NEWCOMBE, CURTIS L. and GOULD, SOPHIA A. Growth indices of *Littorina irrorata*. Part II. Width, shell weight and dry weight of body parts. Biol. Generalis, 13 : Pt. 2. 1937.
5. NEWCOMBE, CURTIS L. and KESSLER, HERMAN. Variations in the growth indices of *Mya arenaria* of the Atlantic coast of North America. Ecology, 17 : No. 3. 1936.
6. PHILLIPS, NORMAN E. and NEWCOMBE, CURTIS L. Growth indices of *Littorina irrorata*. Part I. Length, width, peristome—width and volume. Biol. Generalis, 13 : Pt 2. 1937.
7. WEYMOUTH, FRANK W. The life history and growth of the Pismo clam, *Tivela stultorum*. Calif. Fish and Game Commission, Fisheries Bull. 7, Cont. 37, pp. 1–120. 1923.

CONCERNING THE USE OF INDIRECT BIOCHEMICAL TESTS FOR THE DIAGNOSIS OF CHRONIC CONTAGIOUS MASTITIS[1]

By C. K. Johns[2] and E. G. Hastings[3]

Abstract

Series of samples taken at consecutive milkings were analyzed to determine the reliability of indirect biochemical tests (chlorides, catalase and pH) for the detection of chronic contagious mastitis. It was found that infected quarters not infrequently yield normal milk while many non-infected quarters yield milk giving definitely abnormal reactions. Furthermore, the reactions to these tests frequently fluctuate widely from milking to milking for both infected and non-infected quarters.

These findings suggest the need of caution in the use of these tests as the basis for diagnosing mastitis infection, especially since the proportion of apparently normal animals showing abnormalities in the secretion is probably much larger in many herds than is generally appreciated.

These studies emphasize the value of examining a series of samples at consecutive milkings in order to obtain a true picture of the condition of a quarter. They also suggest that of the three tests studied, the catalase test appears to be the most sensitive indicator of infection.

The diagnosis of chronic contagious mastitis has received a great deal of attention during recent years, and numerous publications record the findings and opinions of workers in this field. Considerable emphasis has been placed by certain workers upon the value of various indirect biochemical tests (chlorides, pH, catalase, etc.) since these are much less laborious and time-consuming than the cultural demonstration of the presence of the streptococcus (*Str. agalactiae*) associated with this disease. Attempts have also been made to compare the accuracy of these indirect tests, although obviously the percentage of positive results from any one test will depend largely upon just where the line is drawn, for any constituent, between the normal and abnormal, as well as upon whether foremilk, middle milk or strippings are examined. The purpose of the present paper is to draw attention to certain objections to the use of these tests as diagnostic agents and particularly to warn against the inadvisability of relying upon such tests, alone or in combination, as the sole basis for diagnosis.

The data to be presented were obtained during studies of abnormal milk from young animals in the herds of the Central Experimental Farm, Ottawa, and the University of Wisconsin, Madison, which by repeated tests had been shown to be free from infection with *Str. agalactiae* or other known pathogens. A few cases of infection with *Str. agalactiae* or *Staph. aureus* were also included for comparative purposes.

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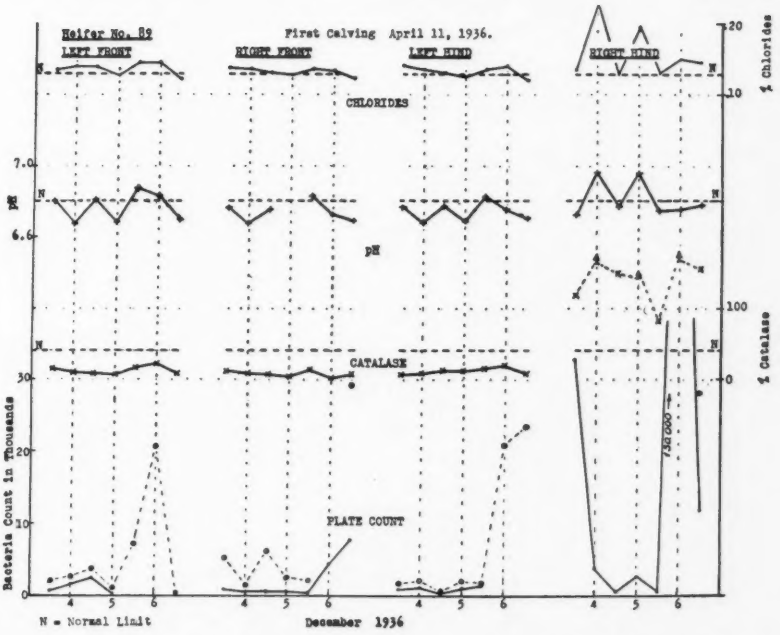

FIG. 1. *Data on foremilk samples from seven consecutive milkings. Heifer No. 89. (Values for morning milkings on vertical dotted lines. Total counts indicated by broken line, counts excluding corynebacteria by solid line.)*

Methods

A number of methods were employed to detect the presence of streptococci, including the use of selective media, overnight incubation of milk with and without brilliant green, etc. Plate counts were originally made upon veal infusion agar but nutrient agar containing 0.5% tryptone (Difco) was mainly used as it yielded better growth.

The pH values were obtained electrometrically using a quinhydrone electrode. Chlorides were determined by titrating 5 cc. of undiluted milk with silver nitrate solution, using dichlorofluorescein as indicator (2). Catalase was determined by the method devised by one of us (E.G.H.); an ordinary glass tube of approximately 8 mm. internal diameter and 300 to 350 mm. length is corked at one end, and melted 2% plain agar introduced to a depth of 1 to 1½ inches. After the agar has solidified, the cork is removed and the agar plug blown one-third to one-half way down the tube. The mixture of milk (2 parts) and one per cent hydrogen peroxide (1 part) is now poured into the tube, the finger being placed at the lower end of the tube to prevent the agar plug from descending further. When the tube is filled, the cork is replaced, the tube inverted and a line marked at the upper level of the liquid. After

incubation for 24 hours at room temperature, the lengths of the columns of gas and liquid are measured and the amount of gas liberated calculated as follows:

$$\frac{\text{column of gas}}{\frac{2}{3}\text{ column of liquid}} \times 100 = \%\text{ oxygen liberated.}$$

Since none of the oxygen liberated is able to escape, as it does in the usual Smith fermentation tube, readings by the latter method are much lower and cannot be satisfactorily compared with those obtained by the method described above. Cheapness, ease of cleaning and a saving of space are other decided advantages of the Hastings tube method.

Normal Values from Infected Quarters

While it is true that a large proportion of animals infected with *Str. agalactiae* yield milk which is high in pH, chlorides and catalase (or cell count, of which catalase is an indirect measure), especially in the first portion drawn, it is likewise true that normal values are not infrequently encountered even when appreciable numbers of streptococci are present. The data recorded for Heifer No. 89 are of interest in this connection. *Str. agalactiae* infection was detected in the right hind quarter during the fifth month of the first lactation period.

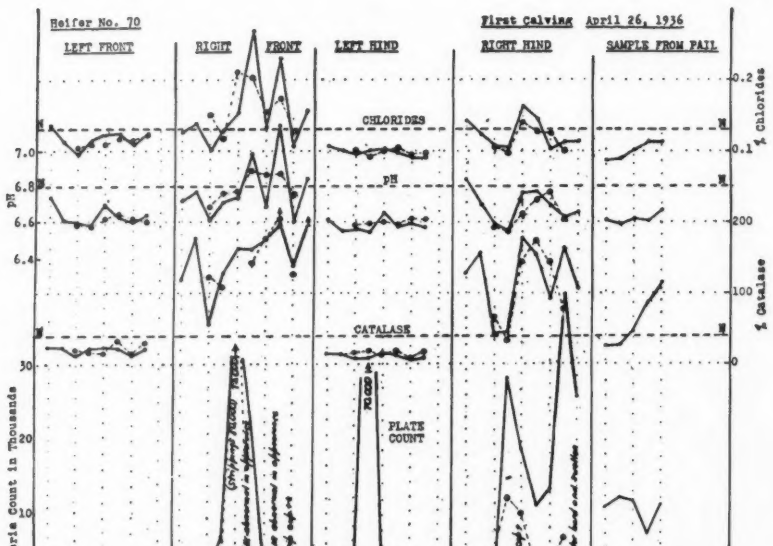

FIG. 2. *Data on foremilk, strippings and pail samples, Heifer No. 70. (Values for afternoon milkings on vertical dotted lines.)*

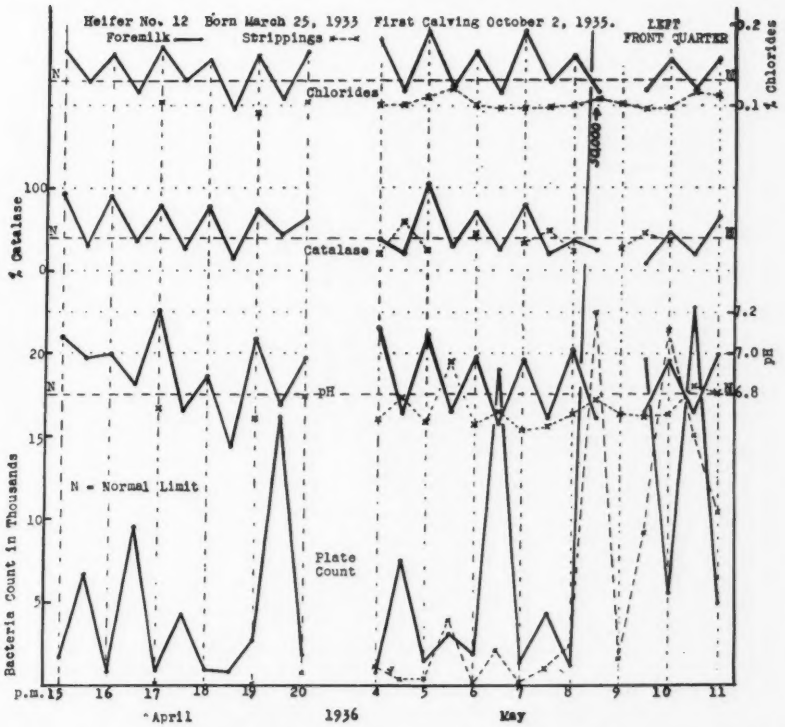

FIG. 3. *Data on foremilk and strippings samples, left front quarter, Heifer No. 12. (Values for afternoon milkings on vertical dotted lines.)*

Analyses of a series of foremilk samples from ten consecutive milkings disclosed little difference between this quarter and the three normal quarters, except for a higher level of values for catalase. A further series of samples from seven consecutive milkings was examined in the eighth month, the data from which appear in Fig. 1. At this time the general levels of values for chlorides and pH, as well as those for catalase, were definitely higher for the infected quarter. It will be observed, however, that there are marked fluctuations in the individual values for this quarter from milking to milking, samples from the afternoon milkings frequently being below the normal limit* for pH. As in the former series, the difference between the infected and normal quarters is most marked with the catalase test, suggesting that this test possesses certain advantages over the chloride and pH tests in the early detection of this type of infection.

* *The limits tentatively established in these studies were: pH (quinhydrone) 6.8; chlorides 0.13%; catalase 40%. These values were found to be substantially equivalent for most of the animals studied.*

Heifer No. 70 was carrying an infection with *Staph. aureus* in both right front and right hind quarters when she was studied during the ninth month of her first lactation period. She suffered an acute attack of mastitis in the right hind quarter on January 15. In Fig. 2 appear data for both foremilk and strippings samples. The contrast between the values for the normal and abnormal quarters is quite striking when a series of samples is examined, but it will be observed that a considerable number of normal values for pH and chlorides were obtained from the two infected quarters, and from the right hind quarter, even at a time when it was obviously hard and swollen. It will be observed that samples from the pail, representing the whole of the milk from the four quarters, were quite normal as judged by the chloride and pH values, while the catalase values increased markedly as the inflammatory process in the right hind quarter became more serious. Here again the catalase reaction appears to be a more sensitive indicator of infection than either the chloride or pH tests.

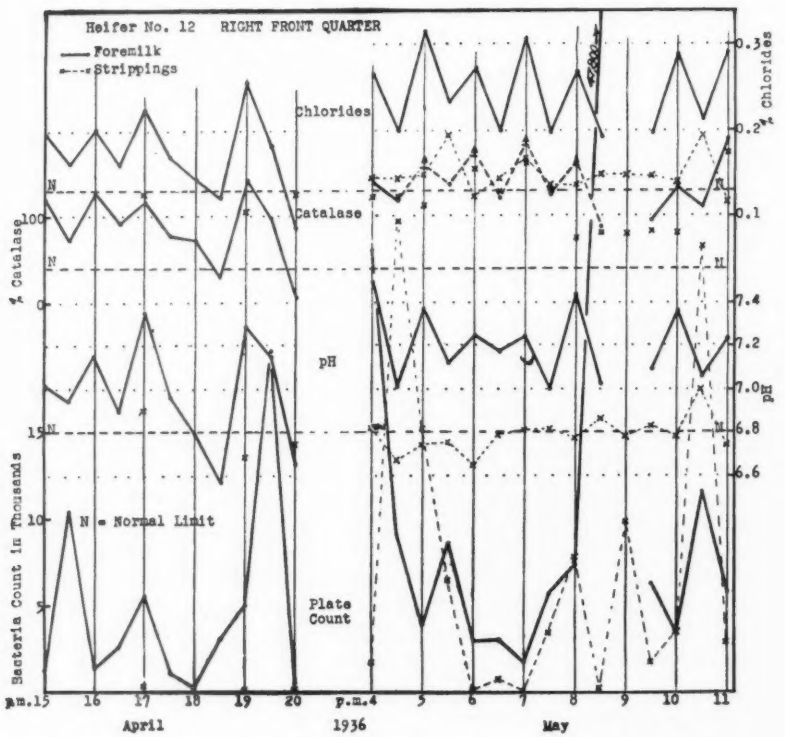

FIG. 4. *Data on foremilk and strippings samples, right front quarter, Heifer No. 12. (Values for afternoon milkings on vertical dotted lines.)*

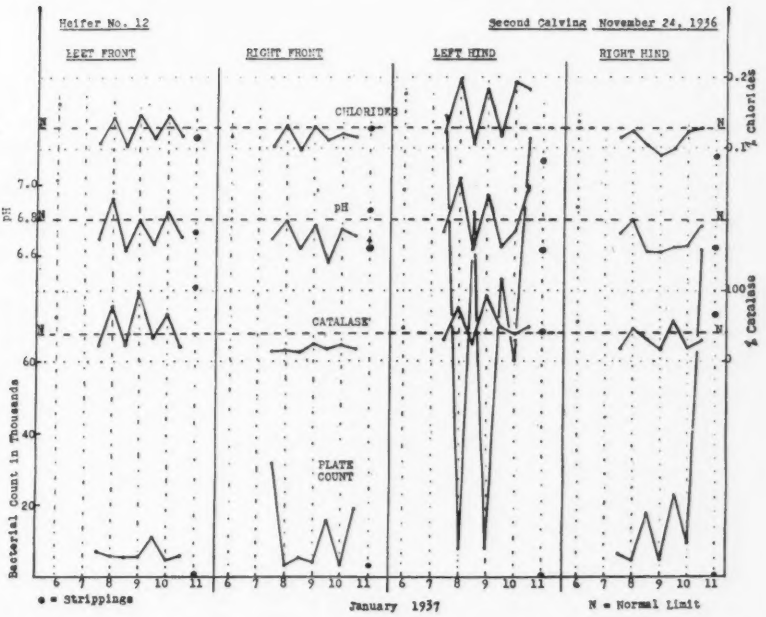

FIG. 5. *Data on foremilk samples, Heifer No. 12. (Values for afternoon milkings on vertical dotted lines.)*

Abnormal Values from Non-infected Quarters

It will be noted that values for chlorides and pH slightly in excess of the normal limits were not infrequently encountered in the samples from the three normal quarters of Heifer No. 89 (Fig. 1). More striking illustrations of the occurrence of abnormal values in milk from quarters free from infection with any recognized pathogen are presented in Figs. 3 to 8. Fig. 3 records data from the left front quarter of Heifer No. 12 during the sixth and seventh months of the first lactation period. Similar abnormal values were encountered for three of the four quarters at this time. Of particular interest are the marked rhythmic fluctuations in biochemical values. Almost without exception the values are abnormal for the afternoon milkings and normal for the mornings, while an inverse relationship is displayed by the bacterial counts. Somewhat similar fluctuations were noted for the right front quarter (Fig. 4) although here the general level of values was definitely higher and few normal values were encountered. Similar pictures were obtained on several other occasions during the first and second lactation periods, of which Fig. 5 is fairly representative.

Another striking example of rhythmic fluctuation in biochemical values is afforded by Heifer No. 94. Samplings at ten consecutive milkings during

the ninth month of the first lactation period yielded the data presented in Fig. 6. Here again the values for the afternoon milkings were almost invariably much higher than those for the morning milkings.

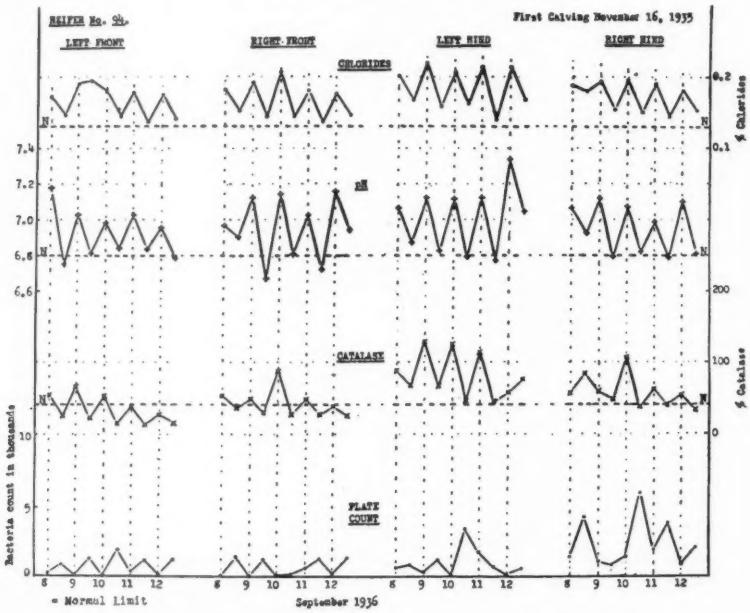

FIG. 6. *Data on foremilk samples from 10 consecutive milkings, Heifer No. 94. (Values for afternoon milkings on vertical dotted lines.)*

Even more marked fluctuations were encountered in the case of Heifer No. 18 (Hart Herd), data from which appear in Fig. 7. The contrast between the values for the normal (left front) and abnormal quarters is particularly striking. While few of the values for the abnormal quarters fall within the the normal limit, it will be observed that there is a marked rhythmic fluctuation, values for the afternoon milkings being almost always higher than those for the morning milkings.

A somewhat different picture (Fig. 8) was obtained from Heifer No. 13 during the second month of her second lactation period. Here the regular rhythmic type of fluctuation is less evident; the general level of values is high, particularly for the front quarters, with tremendous variations from milking to milking. Here again normal values are not infrequently met with, especially on samples from the morning milkings. It will be noted that the values for all four quarters show a fair measure of agreement in their fluctuations.

Discussion

The data presented above represent only a small portion of those obtained during the present studies. Just what proportion of apparently normal

animals in the average herd show abnormalities in the secretion cannot be estimated, but the findings in the Wisconsin Pasture Project herd reported by Hastings and Beach (1), together with those from further studies with other herds there, indicate that the proportion is not inconsiderable. That such abnormalities are unlikely to be due to *latent* infection with *Str. agalactiae* is shown by the following facts. More than seven hundred samples of foremilk, middlemilk and strippings from Heifer No. 12 were examined by a number of different procedures without once demonstrating the presence of the organism, while it has been repeatedly demonstrated without difficulty in samples taken concurrently from definitely infected animals. More than three thousand samples were similarly examined by Hastings and Beach without finding the streptococcus. The Main Herd at the University of Wisconsin has been free from infection with *Str. agalactiae* for more than two years, yet a survey made by one of us (C.K.J.) in March and April, 1937, showed that 39 of the 175 quarters sampled (or 21 of the 44 animals) gave abnormal reactions in at least one out of three samples taken at consecutive milkings. Miller (3) has reported similar findings from first-calf heifers showing no evidence of streptococcus infection. It seems likely therefore that this type of secretion abnormality, in which no recognized pathogen appears to be concerned, may be much more widespread than is generally

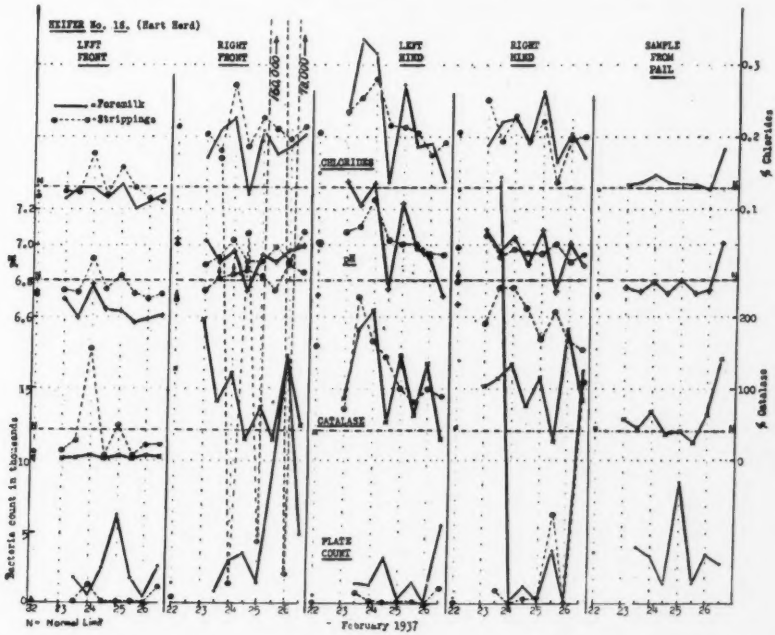

FIG. 7. *Data on foremilk, strippings and pail samples, Heifer No. 18, (Hart Herd) (Values for afternoon milkings on vertical dotted lines.)*

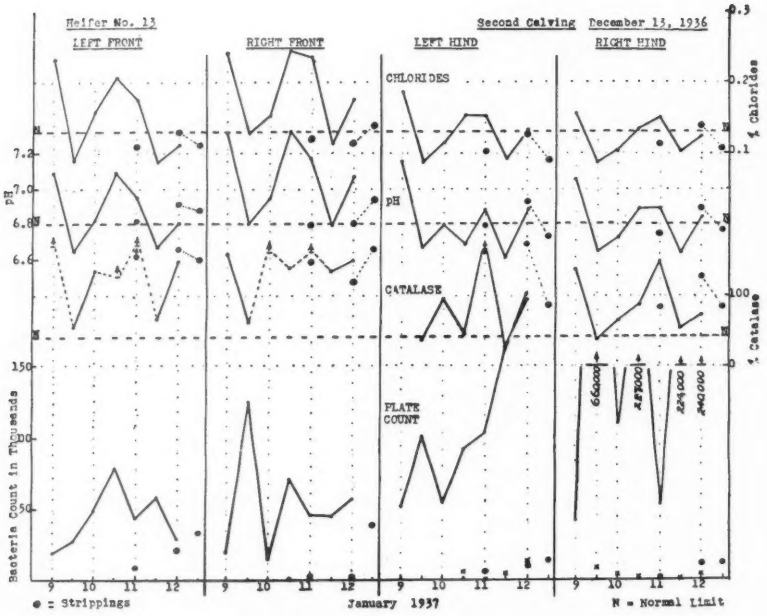

FIG. 8. *Data on foremilk and strippings samples, Heifer No. 13. (Values for afternoon milkings on vertical dotted lines. Counts, excluding corynebacteria, indicated by crosses.)*

realized, and suggests the need for greater caution in the use of indirect biochemical tests as the basis for diagnosing mastitis infection.

A striking feature of the present studies has been the demonstration of marked fluctuations, often of a regular rhythmic character, in the biochemical values for foremilk from milking to milking. Such fluctuations indicate the difficulty of attempting to determine the condition of a quarter by applying any type of test to a set of samples from a single milking. Obviously the verdict in many cases will depend entirely upon whether the samples were taken from the morning or afternoon milking. Similar fluctuations are frequently observed with the bacterial counts. Often low counts occur in samples with high biochemical values and high counts in samples with low values. It is therefore evident that a satisfactory picture of the condition of a quarter can best be obtained by the study of a series of samples taken at consecutive milkings, particularly where an attempt is being made to determine the relation between bacterial numbers and biochemical values.

References

1. HASTINGS, E. G. and BEACH, B. A. J. Agr. Research, 54 : 199-220. 1937.
2. KOLTHOFF, I. M., LAUER, W. M., and SUNDE, C. J. J. Am. Chem. Soc. 51 : 3273-3277. 1929.
3. MILLER, W. T. N. Am. Vet. 17 : 32-41. 1936.